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كلية الطب

**دراسة لبعض الخصائص للبكتريا
المعزولة من حالات الإصابة بالتهاب
الإذن الوسطى في محافظة بابل**

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**University of Babylon
College of Medicine**

**Study of Some Characteristics of the
Bacteria isolated from Patients with
Otitis Media in Babylon Province**

A Thesis

**Submitted to the Council of the College of Medicine - University
of Babylon In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In Medical Microbiology**

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا

إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

صَافٍ اللَّهُ الْعَلِيُّ الْعَظِيمُ

سورة البقرة

الآية (32)

Abstract

One hundred otitis media (OM) swabs were collected from 100 patients (54 of them were females and 46 were males) who were referred to Hilla Teaching Hospital (ENT unit) during a period (from November 2006 through March 2007) suffering from OM. The collected samples were investigated for bacterial isolation.

The ages of the patients were ranged from 7 months to 70 years. The results indicated that, the most age group being susceptible for OM were less than ten years old.

Bacterial culture were positive in (95%) patients versus (5%) patients revealed negative bacterial culture. The most common type of bacterial isolated were *Moraxella catarrhalis* (20.83%), followed by *Pseudomonas aeruginosa* (20%), *Streptococcus pneumoniae* (19.6%), *Staphylococcus aureus* (17.5%), *Klebsiella pneumoniae* (5%), *Proteus* and *Acinetobacter* (4.16%), *Staphylococcus epidermidis* (3.3%), *Streptococcus pyogenes* and *E. coli* (1.66%) one isolates of *Haemophilus influenzae* and one for *Diphtheroidis* spp (0.83%). Under anaerobic condition only one bacteria was isolated belonging to *Bacteroides* spp was (0.83%).

Besides, uronic acid was detected in most samples of ear discharge, taken under study, and this acid was found to be associated with most isolates, especially *K. pneumoniae*, *S. pneumoniae* and *P. aeruginosa* infections, whereas this acid was not observed in discharge with *M. catarrhalis*.

The presence of capsule and its size have effect on the amount of the uronic acid produced by the bacteria. The bacteria with small capsule have a little amount of acid production.

The virulence factors of some bacteria isolated in this study were studied. The capsule was present in all *K. pneumoniae* isolates (100%) and in (60%) of *P. aeruginosa* and *S. pneumoniae* isolates, whereas (10%) of *S. aureus* isolates had

capsule, the other bacteria such as *M. catarrhalis* isolates in this study did not possess the capsule.

Colonization factor antigens were detected in all isolates of *S. pneumoniae*, and (80% and 75%) of *M. catarrhalis* and *K.pneumoniae* isolates respectively, whereas (50%) of *P. aeruginosa* isolates and (40%) of *S. aureus* isolates having the ability to produce colonization factors.

The results also showed that all isolates of *K. pneumoniae* and *M. catarrhalis* did not produce hemolysin, while all *S. pneumoniae* and *S. aureus* isolates were able to produce hemolysin, and only (30%) of *P. aeruginosa* isolates were able to produce hemolysin.

All isolates of *S. pneumoniae* and *S. aureus* were not able to produce siderophores, but all *K.pneumoniae* and *P.aeruginosa* isolates were able to produce siderophores, and only (40%) of *M. catarrhalis* isolates were able to produce siderophores.

Extracellular proteases were also investigated . It was found that 75% isolates of *K. pneumoniae* isolates were able to produce extracellular proteases, whereas (30%) of *P. aeruginosa*, (20%) of *S. aureus* isolates and *M. catarrhalis*, 16.6% of *S. pneumoniae* isolates had the ability to produce this enzyme.

The effect of some antibiotics on some bacterial isolate has been studied. It has been found that Clarithromycin and Ciprofloxacin are the most effective antibiotics towards the tested bacterial isolates, whereas Doxycyclin and Amoxicillin are the least effective.

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I Extend my thanks to the medical staff in Hilla Teaching Surgical Hospital unit (ENT)for helping me in the collection of samples.

Nada

Appendix (1)

Morphological and Biochemical Features for Identification of Gram Negative Isolates

Test	<i>M. catarrhalis</i>	<i>P. aeruginosa</i>	<i>Proteus</i>	<i>K. pneumoniae</i>	<i>Acinetobacter</i>	<i>E.coli</i>
Gram stain	G-ve- - (Diplococci)	G-ve,- short rods	G-ve,- short rods	G-ve,- short rods	G-ve- Coccobacilli (Diplococci)	G-ve, - short rods
Catalasase	+	+	+	+	+	+
Oxidase	+	+	-	-	-	-
Capsule	-	-, +	-	+	-	-
Hemomysin	-	+, -	-	-	-	-
Indol	-	-	-	-	-	+
V.P.	-	-	-	+	-	-
Cimon citrate	-	-	+	-	+	+
M.R.	-	+	-	+	+	-
Growth on KIA	-	K/K	k/a	A/a	k/k	A/a
H ₂ S production	-	-	+	-	-	-
motility	-	+	swarming	-	-	+, -

*The major diagnostic test for *P. aeruginosa* is the production of blue(pyocyanin) pigment with the yellow / green pyoverdin(fluorescein) giving the characteristic blue-green appearance of culture.

Appendix (2)

Morphological and Biochemical Features for Identification of Gram Positive Isolates

Test	<i>S.pneumoniae</i>	<i>S.pyogenes</i>	<i>S.aureus</i>	<i>S.epidermidis</i>
Gram stain	G+ve + diplococci	G+ve + cocci (chains)	G+ve cocci (clusters)	G+ve cocci (clusters)
Catalase	-	-	+	+
Oxidase	-	-	-	-
Hemomysin	Alpha	Beta	Beta	Gama
coagulase	-	-	+	-
Growth on mannitol	-	-	Golden	White
motility	-	-	-	-
Bacitracin sensitivity	Resist	Sensitive	/	/
optochin sensitivity	Sensitive	Resist	/	/
Bil soulbilty	Sensitive	Resist	/	/

Appendix (3)

The colonic acid production by using Galactouronic acid

Galactouronic acid in concentration 100% micromole / ml	D.W.	The final concentration	Optimal density
1 ml	9 ml	100 Mg / ml	1.6
0.8 ml	9.2 ml	80 Mg / ml	1.3
0.6 ml	9.4 ml	60 Mg / ml	1.0
0.5 ml	9.5 ml	50 Mg / ml	0.8
0.4 ml	9.6 ml	40 Mg / ml	0.65
0.2 ml	9.8 ml	20 Mg / ml	0.4
0.1 ml	9.9 ml	10 Mg / ml	0.23

Certification

We certify that this thesis was prepared under our supervision at the Department of Microbiology, College of Medicine, University of Babylon as partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

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In view of the available recommendation, I present this thesis for evaluation by the Examining Committee.

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We, the examiner committee, certify that we have read the thesis entitled **(Study of some characteristics of the bacteria isolated from Patients with Otitis Media in Babylon Province)** and have examined the student **(Nada Khazal Kadhim Hendi Alwae'li)** in its contents, and that in our opinion it is accepted as a thesis for the degree of Master of Science in Medical Microbiology with excellent estimation.

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Conclusions & Recommendations

Conclusions

According to this study, we can conclude the following :

1. The types of bacteria which causes otitis media from the most to least common are *M.catarrhalis* , *P.aeruginosa* ,*Strep. pneumoniae* and *Staph. aureus*.
2. Anaerobic bacteria (*Bacteroidis spp*) were also present and isolated from one case.
3. All bacteria isolates in this study have the ability to possess more than one virulence factors such as capsule, siderophors, hemolysin, extracellular protease, and adherence factors to produce otitis media infection .
4. The high concentration of uronic acid increased with large size of capsular polysaccharides arounded the colonies of bacteria .
5. The best drug of choice in the treatment of otitis media are ciprofloxacin and clarithromycin.
- 6.The peak of incidence of otitis media occurs in the age group of <1-10 years and less frequent adult .
- 7.There is no significant variation between males and females infected with otitis media .

Recommendations

According to this study, we can recommend the following :

1. The early treatment of URTI in children, because the URTI lead to produce otitis media.
2. The use of new antibiotic should be highly selective and is not used for long time to decrease the chance of emergence of bacteria drug resistance.
3. Introduction of new molecular techniques (using PCR) for detection of fastidious microorganism or viruses associated with otitis media other than bacteria is recommended.

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Abbreviations

Symbol	Description
DW	Distilled water
ENT	Ear nose throat
MRSA	Methecilin Resistant <i>Staphylococcus aureus</i>
OM	Otitis Media
CSOM	Chronic Supportive Otitis Media
URTI	Upper respiratory tract infection
ME	Meddle Ear
ET	Eustachian Tube
TM	Tympanic Membrane

Dedication

*To the Daughter of the Holy Prophet and the Wife of
Imam Ali*

Fatima Al-Zahra

Leader of the women of paradise

And To my mother, my father

To my brothers ,my sisters with my endless love

الخلاصة

تم خلال هذا البحث جمع و دراسة 100 عينه (مسحات اذن وسطى) من 100 مريض (54 منهم إناث و 46 ذكور) مصابين بالتهاب اذن وسطى (حسب التشخيص السريري الاولي من قبل اختصاصي الأنف والأذن والحنجرة) و ممن أحيلوا إلى مستشفى الحلة التعليمي العام (من تشرين الثاني 2006 خلال اذار 2007) . تراوحت أعمار المرضى الذين شملتهم الدراسة من 7 اشهر - 70 سنة .
اظهرت النتائج أن أكثر فئة عمرية تعرضا لالتهاب الاذن الوسطى هي الفئة العمرية اقل من 10 سنة .

كانت نتائج الزرع موجبة ل 95 مريض مقابل 5 نتائج سالبة اتجاه الزرع . الأنواع البكتيرية التي تم عزلها وتشخيصها خلال هذه الدراسة شملت أنواعا بكتيرية موجبة وسالبة لصبغة غرام جاءت في مقدمتها بكتريا *Moraxella catarrhalis* حيث شكلت نسبة مئوية مقدارها 20.83% ، جاءت بعدها بكتريا *Pseudomonas aeruginosa* (20%) ، ثم *Streptococcus pneumoniae* (19.6%) ثم *Staphylococcus aureus* (5.17%) ، ثم الاقل شيوعا *Klebsiella pneumoniae* (5%) ثم *Acinetobacter and Proteus* (4.16%) ثم *Staphylococcus epidermidis* (3.3%) ، ثم *E.coli* و *Streptococcus pyogenes* (1.66%) و عزلة واحدة من بكتريا *Haemophilus influenzae* و *Diphtheriodis* (0.83%) . وأخيرا عزلة واحدة من البكتريا الاهوائية كانت *Bacteroides spp* (0.83%) . وكذلك تضمنت هذه الدراسة حامض اليورونك من افرازات الاذن الوسطى .

اظهرت النتائج بان اغلب العزلات المنتجة للمحفظة كانت (*S.pneumoniae* و *K.pneumoniae* و *P.aeruginosa* و *S. aureus*) تستطيع ان تنتج حامض اليورونك بينما *M.catarrhalis* لا تستطيع ان تنتج هذا الحامض لانها لا تحتوي على المحفظة .
كما ان كمية الحامض المنتجة من قبل البكتريا المكونة للمحفظة تتأثر بحجم المحفظة , كلما كان حجم المحفظة كبير كانت كمية الحامض المنتجة كبيرة والعكس صحيح .
كما تم الكشف عن بعض عوامل الضراوة التي تمتلكها هذه العزلات البكتيرية لكي تؤهلها للتسبب التهاب الاذن الوسطى , وقد أظهرت النتائج بأن :

اظهرت جميع عزلات بكتريا (*M. catarrhalis*) غير حاوية على المحفظة ، في حين جميع عزلات *K. pneumoniae* كانت حاوية على المحفظة . وكذلك وجد بأن

60 % من عزلات (*S. pneumoniae* و *P. aeruginosa*) كانت حاوية على المحفظة ، و (10%) فقط من عزلات (*S. aureus*) حاوية على المحفظة .
تمّ الكشف عن قابلية هذه العزلات البكتيرية على امتلاك عوامل الاستيطان ، ووجد أن جميع عزلات (*S. pneumoniae*) تمتلك عوامل الاستيطان . إن 80% من عزلات (*M. catarrhalis*) وكذلك 75% من عزلات (*K. pneumoniae*) في حين وجد أن 50% من عزلات (*P. aeruginosa*) و 40% من عزلات (*S. aureus*) تمتلك عوامل الاستيطان .

جميع عزلات (*M. catarrhalis*) و (*K. pneumoniae*) لم تظهر القابلية على إنتاج الهيمولايسين ، في حين جميع عزلات (*S. pneumoniae*) و (*S. aureus*) تمتلك القدرة لإنتاج الهيمولايسين . وكذلك وجد أن (30%) فقط من عزلات (*P. aeruginosa*) تمتلك القدرة لإنتاج الهيمولايسين .
كل عزلات (*S. pneumoniae*) و (*S. aureus*) غير قادرة على إنتاج السايديروفورات ، ولكن جميع عزلات (*P. aeruginosa*) و (*K. pneumoniae*) كانت قادرة على إنتاج السايديروفورات و (40%) فقط من عزلات (*M. catarrhalis*) كانت قادرة على إنتاج السايديروفورات .

75 % من عزلات *K. pneumoniae* تمتلك القابلية على إنتاج أنزيمات البروتياز الخارجية ، و وجد أن 30% من عزلات (*P. aeruginosa*) و (20%) من عزلات (*M. catarrhalis* و *S. aureus*) لها القابلية على إنتاج أنزيمات البروتياز الخارجية ، في حين أن (16.6%) من عزلات (*S. pneumoniae*) تمتلك القابلية على إنتاج أنزيمات البروتياز الخارجية .

جرت دراسة تأثير المضادات الحيوية على بعض العزلات البكتيرية. وقد تبين من خلال هذه الدراسة بأن المضادين الحيّاتيين السبروفلوكلسلسين وكلاريثروميسين هما الأكثر تأثيراً على كل العزلات البكتيرية بينما كان المضادان الحيّاتيان: الدوكسيسيكليين والاموكسيلين هما الأقل تأثيراً .

1-1.Introduction :

Otitis media (OM) means inflammation of the middle ear (ME) it constitutes the most common respiratory tract infection of infancy and early childhood (Kenna,1994; Little *et al.*,2006) .

Otitis media may be acute,or chronic suppurative type.Children are mostly affected with acute type ,while adults are mostly affected with chronic suppurative types.(Arroll,2005).

The infection of otitis media might result from viral or bacterial agents, however many complications were reported that may persist in some individuals in to adult years (Little,*et al.*,2006) .

The bacteriologic agents associoted with otitis media differ according to the type ; *S.peumoniae* ,non typable *H. influneza* , and *M. catarrhalis* are most common bacterial of acute OM (Lee,2003) ,*P. aeruginosa*, *S.aureus* ,and *Proteus spp.* are predominant pathogen of chronic suppurative otitis media,and also it was seen that non spore forming anarebes such as *Bacteroides* spp can be isolated from exudates obtained from patients with chronic otitis media (AL- Dahhan *et al.*,2005).

Pathogenic bacteria have the ability to produce several types of virulence factors associated with their pathogenicity and major role in the causation of infection in otitis media, the main virulence factors are capsule ,hemolysine, siderophores, proteases,and colonization factor antigen (CFA).

The epidemiology of otitis media varies in different populations according to the influencing factor ; which includes sex, age, climates, genetic factors, exposure smoking, environmental factors and upper respiratory tract infections (URTI) (Aliphas *et al.*,2006).

Otitis media in Iraq in all types are recorded and the prevalence of it differs according to the types of otitis media, and also patients location , sex and occupation.

Aims of this study To:

1. Isolate and identify the common bacterial pathogens associated with otitis media .
2. Detection of uronic acid from ear discharge.
3. Examine some virulent factors such as (capsule, hemolysin, siderophores, extracellular proteases, and colonization factor antigen).
4. Show the effect of some Antibiotics on common bacterial isolates.

1-2. Literature Review

1-2-1 Anatomy of the Ear:

The ear has three major parts; the outer ear (OE), middle ear (ME), and inner ear. The outer ear includes the pinna and External auditory canal (EAC), and extends to the tympanic membrane (TM) which separates the (OE) from the ME. The ME is an air chamber which contains three tiny bones; the malleus, incus, and stapes. The ME is connected to the nasopharynx area through Eustachian tube (ET). Inner ear contains the hearing and balance organs (cochlea) (Scott Browns, 1987, and Barbara and Homeier, 2005).

1-2-2 Definition and Classification of Otitis Media:

The otitis media is an inflammation of the mucous membrane lining the ME cleft, produced by pus-forming organism (Scott Brown, 1987). OM is very common in infant, and children age 3 months to 3 years, and also prevalent in adults (Gates, *et al.*, 1994).

The symptoms of OM may include ear pain, fever, aural fullness, hearing loss, irritability, pulling of the ear and sometimes diarrhea and vomiting (Greene, 2002).

1.2. 2.1. Types of Otitis media

There are several kinds of Otitis media

1. 2.2.1.1. **Acute Otitis media:** AOM refers to the infection of the ME with sudden onset and short duration, the symptoms of acute OM may include pain, redness of the ear drum and possible fever (Rockville and Bethesda 2006). *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, and

Gram's negative enteric bacteria represent the typical pathogens of acute OM (Marchett ,*et al.*,2005).

1.2.2.1.2. Otitis media with effusion (OME):

Formerly termed serous OM or secretory OM, is middle ear effusion of any duration that lacks the association with signs and symptoms of infection(eg. fever, otalgia , irritability).OME usually follows an episode of acute OM .The fluid in OME is often thin and watery. Glue ear is a common name given to OME with thick viscous effusion.OME in which there is an intact TM with fluid in the ME but no active infection (Rockvill and Bethesde, 2006).

1.2.2.1.3.Chronic Otitis Media (COM):

Is characterized by recurrent or persistent ear discharge (otorrhea) over (2-6weeks), through a perforation of the TM ,COM occurs when the ET becomes blocked repeatedly due to allergies, multiple infections,ear trauma or swelling of the adenoids (Brunton and Pichincher, 2005).COM may be suppurative or non suppurative , chronic suppurative otitis media (CSOM) refers to chronic ME infection with non healing perforation of TM , discharge presents in the active state of disease, CSOM is a form of OM which is still one of the commonest medical problems, some studies defined this illness as a persistent disease, insidious in onset, often capable of causing severe destruction and irreversible sequelae, Clinically manifested with deafness and discharge .Gram negative bacilli, particularly *P.aeruginosa*,*Proteus* spp and anaerobes were the predominant causative pathogens for this disease (Macnamar *et al.*,1999 and Lee, 2003).

1. 2.2.1.4. Other types of Otitis media:

A. Adhesive Otitis media

B. Tuberculosis Otitis media:

The ear rarely infected by M tuberculosis , current incidence is estimated to be between 0.9% and 0.04. The Tubercle bacillus can spread to the ME by several routes , the most common route is hematogenous, can spread through the ET. Tuberculous OM with painless, multiple TM perforation, abundant pale granulation tissue in ME and early severe hearing loss (Callender, 1992).

1.2.3 Routes of infections :

A. Eustachian tube (ET).

B. External auditory canal (EAC).

C. Blood borne infection (Scott Browns, 1997).

1.2.4 Predisposing factors of Otitis Media

1.2.4.1. Age and Sex:

Otitis media whether in suppurative or non suppurative, acute or chronic type, affects both sexes of different ages and higher incidence was found younger age (Finkelstein *et al.*, 2005). Otitis media is an early children disease (children below the age of seven years are much more prone to OM since the ET is shorter, wider and of a horizontal angle than in the adult ear, the ET might allow easier access of bacteria from the nasopharynx to the ME) (Ilicali, *et al.*, 1999).

Shimotakahara *et al.*, (1998) reported that there is no significant

differences in the incidence of OM in relation to sex as both sexes were equal to infection .

1.2.2.4. Eustachian tube malfunction:

Eustachian tube (ET) connects the ME with nasopharynx, disfunction of the ET appears to play the development role of fluid and infection of the middle ear, congestion of the mucosa of ET may result in obstruction secretion , and if a bacterial pathogen is present a suppurative otitis media may result (Klein *et al.*, 2000). Odemas formation of the ET may result obstruction of it (Tarlow, 1998).

1.2.4.3. Upper respiratory tract infection (URTI) :

Many patients with state that their ear starts to discharge after an URTI. Chronic rhinitis and sinusitis produce a constant flow of infection mucous which may enter the ET . The viral infection would also affect the mucosa of the ME making it less resistant to the organisms that are normally in the ME, allowing bacterial over growth (Fireman, 1997).

1.2.4.4. Environmental factors (socioeconomic factors):

The incidence of otitis media is highest in low hygiene , over crowding and malnutrition, significant association with acute OM were found by Adderson (1998).

1.2.4.5. Other factors:

Day care, passive smoking, swimming, breast feeding, genetic and racial factors, allergy immunodeficiency (β cell deficiency) , and seasonal variations are aetiological factors in otitis media (Argueds, *et al.*, 2006).

1.2.5 Microbiology of otitis media

1.2.5.1. Bacterial infection

It was focused on the top of five ear potential bacterial pathogens as in the following :

1.2.5.1.1. *Streptococcus pneumoniae*:

It is a gram positive, lancet shaped, it is seen in pairs, diplococci, but it also occurs singly and in short chains. It is alpha(α) hemolytic when cultured on blood agar, but can cause β hemolysis during anaerobic conditions. It is a non motile, non spore forming, fastidious, growing best in 5% carbon dioxide, fermentative aerotolerant anaerobe (Brook,et al.,2004).

Streptococcus characterized by a lack of cytochromes and catalase, growth requires a source of catalase (eg. Blood) to neutralize the large amount of H_2O_2 produced by the bacteria (Austrian,et al.,1998) *S.pneumoniae* exists in 2 forms;capsulated and noncapsulated strain.Special tests for identification this bacterium such as inulin fermentation ,bile solubility and optochin sensitivity (Brook et al.,2004) *S.pneumoniae* is very fragile bacterium and contains within itself the enzymatic ability to disrupt and to disintegrate the cells, the enzyme is called autolysin (Todar, 2003).

1.2.5.1.1.1. *pathogenicity*:

S. pneumoniae is normally found in the nasopharynx of 5-10%of healthy adults and 20-40%of healthy children (Peterson,2006).It attaches to the nasopharyngeal cells through interaction of bacterial surface adhesines and epithelial cells,this normal colonization can become infection

if the organisms are carried into areas, such as ET or nasal sinuses where it can cause OM and sinusitis respectively. Pneumonia occurs if the organisms are inhaled into the lungs. The organism spreads to the blood stream (where it can cause bacteraemia) and is carried to the meninges, joint spaces, bones and peritoneal cavity, and may result in meningitis, brain abscess, septic arthritis or osteomyelitis. *S. pneumoniae* may cause epidemic bacterial conjunctivitis. (Hanniffy, *et al.*, 2007). The pathogenicity of *S. pneumoniae* has been attributed to various structures, most of which are situated on its surface. The best defined virulence factor which plays an important role in pathogenicity is the polysaccharide capsule which provides resistance to phagocytosis and thus promotes the escape of pneumococci from the host immune defense, other factors including cell wall components and the intracellular toxin pneumolysin, are involved mainly in the inflammation caused by infection (Musher, 1992). The risk of pneumococcal infection is much increased in persons with impaired immunoglobulin synthesis (Lysenko *et al.*, 2005).

1.2.5.1.1.2. *Virulence factors:*

S. pneumoniae expresses different virulence factors on its cell surface and inside the organism. These virulence factors during infection with *S. pneumoniae* include;

1.2.5.1.1.2.1. *Capsule :*

The capsular polysaccharide is the major virulence factor of *S. pneumoniae* the capsule is antiphagocytic, inhibiting complement deposition and phagocytosis where type specific opsonic antibody is absent, (Kilian, 2002), during invasion, the capsule is an essential determinant of

virulence .The capsule interferes with phagocytosis by preventing c3b opsonization of the bacterial cells . Ninety different capsule types of pneumococci have been identified and form the basis of antigenic serotypes of the organism (Alosodevelasco,*et al.*, 1995) *S .pneumoniae* have the ability to transfer cassettes of capsular genes from one strain to another leading to a change in capsule specificity, all have potential , for vaccine strategy the first gene of the capsular polysacchride biosynthesis locus (cp52A),(Tuomanon *et at* ,. 1995) .

1.2.5.1.1.2.2 *Extracellular proteases:*

Pnemococci produce an extracellular proteases that specifically cleaves human IgA1 in the hinge region into fab and fc fragments. This protease enables these bacteria to evade the protective functions of the principal immunoglobulin isotype of the URT(Gorbach *et al.*,1998). Anti IgA1proteases have been implicated as potentially important, virulence factors produced by a variety of human bacterial pathogens. Most of these organisms are known to be pathogenic for only human beings and don't cause significant naturally occurring disease in animal species (Proctor and Manning , 1999; and McDaniel and Larry, 2005).

1.2.5.1.1.2.3 *Adhererence factors :*

S.pneumoniae expresses two antigens I/II family, binding to fibronectin is essential for the attachment of *S. pneumoniae* respiratory endothelial cells. This binding activity is mediated by several fibronectin binding proteins included in this group are PsaA from *S. pneumoniae* (Wizemann,*et al.*,1999).

The *S.pneumoniae* homologue of FimA, PsaA, also has a role in pathogenesis while it commonly colonizes the nasopharynx of healthy person. *S. pneumoniae* is common pathogen in children and older adults and a leading causes of otitis media (Novak *et al.*, 1998).

1.2.5.1.1.2.4 *Pnemolysin and Siderophores:*

Pneumococci produce an intracellular membrane damaging toxin known as Pnemolysin which release by autolysis (Pnemolysin is released up on lysis of *Pneumococci* by autolysin) . Pnemolysin inhibits (neutrophil chemotaxis ,phagocytosis and the respiratory burst , lymphocyte proliferation and immunoglobulin synthesis) . Pnemolysin is immunogenic and it might be suitable for a new *Pneumococcal* vaccine . Pnemolysin(ply) protein that can cause lysis of host cells and activate complement (Hanniff, *et al.*,2007).

Siderophores production was not detected by either chemical or biological methods.Its utilization of iron containing compounds found in human hosts was tested. Both lactoferrin and transferrin failed to do so hemin in a culture lacking other iron source (Tai, *et al.*, 1993).

1.2.5.1.2 *Staphylococcus aureus:*

S. aureus is a Gram's positive, spherical in shape, it is single or in clusters, and occasionally in short chains. It is a non-motile, non-spore-forming, facultative anaerobe; it is catalase-positive, oxidase-negative, and coagulase-positive usually non capsulare colonies appear opaque and are often pigmented golden yellow . In common with other facultative

anaerobes, *S.aureus* can grow in the absence of oxygen by has both fermentation and oxidative pathways (Masalha, *et al.*, 2001).

Several studies suggest that oxygen plays a role in the pathogenesis of *S .aureus* in both its capacity to produce virulence factors and its ability to persist and grow in different and often hospital environmental niches (Yarwood and Schlievert, 2000).

Methicillin resistant *S.aureus* become increasing singly problematic due to the emergence of resistant strain (Murray *et al.*, 2003).

1.2.5.1.2. 1. *Pathogenicity* :

S. aureus is present in the nose of 30% of healthy people and URT, and may be found on the skin (Green wood, *et al.*, 2002)

S. aureus express many potential virulence factors such as: surface proteins that promote colonization of host tissues, factors that probably inhibit phagocytosis (capsule, immunoglobulin binding protein A), toxins that damage host tissues and cause disease symptoms (Archer, 1998).

S. aureus the major causative agent of hospital acquired (nosocomial) infection of surgical wound and epidermal skin diseases in newborn infants and food poisoning (Brook *et al.*, 2004). Also, *S. aureus* can cause septicaemia, folliculitis, boil, scalded skin syndrome, conjunctivitis, osteomyelitis, endocarditis, and mastitis (Schneewind, *et al.*, 1995, and Chigbu and Ezeronye, 2003).

S.aureus is the most common cause of bacterial otitis media world wide (Ikeh *et al.*, 1993, and ALDahhan, *et al.*, 2005). The pathogenicity of *S. aureus* contributes to hemolysis of blood, coagulation of the plasma and

production of intracellular enzyme and toxins which act on host cell membrane and mediate the cell destruction (Brook *et al.*, 2004).

1.2.5.1.2. 2. *Virulence Factors*

1.2.5.1.2. 2.1 *Capsular Polysaccharide :*

S.aureus isolates can produce one of 11 different capsular serotypes. Serotypes 5 and 8 are the predominant which account for about 80% of isolates (Arbeit, *et al.*, 1984).

The capsule has been called a microcapsule .It may impede phagocytosis ,but in vitro tested this was only demonstrated in the absence of complement. Conversely, comparing wild type and a capsule defective mutant strain in an endocarditis model suggested that polysaccharide expression actually impeded colonization of damage heart valves,perhaps masking adhesins(Foster,*et al.*, 1994).

1.2.5.1.2. 2.2 *Extracellular Proteases :*

Protein A is surface Protein of *S. aureus* which binds IgG molecules by the Fc region, in serum, bacteria will bind IgG molecules the wrong way round by non immune mechanism, in principle this will disrupt opsonization and phagocytosis (Todar, 1998) indicating that they are important virulence factors. Recent reports have suggested that proteases also play a role in the transition of *S. aureus* cells from an adhesive to an invasive phenotype by degrading bacterial cell surface proteins, such as fibronectin binding protein and protein A (Karlsson, *et al.*, 2001).

S. aureus produces four major extracellular proteases: serine protease, a cysteine protease, metalloprotease and a second cysteine protease (also named staphopain) (Karlsson and Arvidson, 2002).

1.2.5.1.2. 2.3 Adherence Factors :

Some of the most well-characterized colonization factors in gram-positive bacteria, the polypeptides of the antigen I/II family. Surface proteins of the antigen I/II family contain alanine-rich repeats (Wizemann, *et al.*, 1999).

S. aureus expresses fibronectin-binding adhesions. Two genes encoding for fibronectin-binding proteins have been identified in *S. aureus*-fnbA and fnbB. Fibronectin binding activity is critical in pathogenesis because it allows the bacteria to adhere to extracellular matrix components including fibronectin and collagen. This can result in cutaneous infections and in life-threatening bacteremia and endocarditis (Schennings, *et al.*, 1993).

1.2.5.1.2. 2.4 other Virulence Factors

I. Hemolysin and Siderophores:

These factors cause membrane damaging toxins that lyse eukaryotic cell membrane. Various degrees of hemolysin produced by *S. aureus*.

S. aureus is often Beta hemolytic on blood agar. (Murray *et al.*, 2003).

S. aureus possesses both siderophore-mediated and nonsiderophore iron uptake systems. Each system plays a role during pathogenesis. Several *S. aureus* strains produce siderophores, two of these siderophores, staphyloferrin A and staphyloferrin B are of the polycarboxylate class,

while the third aureochelin is chemically uncharacterized (Dale, *et al.*, 2004). *S. aureus* may be able to scavenge different various sources of host iron (e.g., heme and hemoglobin) during the establishment of an infection, and indeed, *S. aureus* does possess the ability to bind heme and hemoglobin involved in the transport of staphylobactin (Mazmanian, *et al.*, 2003).

II. Coagulase:

S. aureus can produce an extracellular enzyme coagulase which convert plasma fibrinogen in to fibrin ,aided by an activator present in plasma (Green wood,*et al.*, 2002).

III. Clumping factor or Bound Coagulase:

Is a surface *S. aureus* compound that is responsible for adherence of organism to fibrinogen and fibrin ,when mixed with plasma .

S. aureus forms clumps, clumping factor is distinct from coagulase. (Green wood,*et al.*, 2002).

1.2.5.1.3. Klebsiella pneumoniae:

K. pneumoniae is a gram-negative, it is a member of the family Enterobacteriaceae, that charcterized by polysaccharide capsule (which give their colonies their characteristic mucoid appearance), rod-shaped, facultative anaerobic. It is a non-motile, non-spore-forming flagellated, produces large sticky colonies when plated on nutrient media (Podschun and Ulmann, 1998).

K. pneumoniae is lactose fermenter, produces lysine decarboxylase but not ornithine decarboxylase and it is generally positive in the voges-proskauer test and citrate, produces gas from glucose (Brook *et al.*, 2004).

1.2.5.1.3. 1. *Pathogenicity* :

Colonization of the respiratory tract by *K. pneumoniae* is very common in hospital patients receiving antibiotics (Green wood, *et al.*, 2002). *Klebsiella* infections are encountered more often now than in past. This is probably due to bacteriums antibiotic resistance properties. In general, *K. pneumoniae* can cause pneumoniae, urinary tract infection, and bacterial conjunctivitis. *K. pneumoniae* is one of the etiologic agents of OM (Krohn, *et al.*, 1993 and Ibekwe *et al.*, 1997).

Klebsiella's pathogenicity may be attributed to its production of a heat stable enterotoxin. The major virulence factor of *K. pneumoniae* which plays an essential role in pathogenesis, is the thick capsule (it's antiphagocytic), and fimbriae (pili) (Podschun, *et al.*, 2001).

1.2.5.1.3. 2. *Virulence Factors*

1.2.5.1.3. 2.1. *Capsule*:

Thick capsule is a major virulence factor of *K. pneumoniae*, which is composed of complex acidic polysaccharides, and form more than 70 serotypes (Green wood, *et al.*, 2002). The capsular repeating subunits consisting of four to six sugars and very often, uronic acids (as negatively charged components). The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers (Amako, *et al.*, 1988). The capsule protects the bacterium from

phagocytosis by polymorphonuclear granulocytes on the one hand, and prevents killing of the bacteria by bactericidal serum factors on the other (Podschun, *et al.*, 1992; and Green wood,*et al.*, 2002).

1.2.5.1.3. 2.2 .*Extracellular Proteases* :

Proteases enzymes are important enzymes that play essential roles in pathogenesis. *K. pneumoniae* strains can not produce extracellular proteases but can depend on other mechanisms for uptaking materials as a need to their growth, such as production of siderophores (Al-Saedi, 2000).

1.2.5.1.3. 2.3 .*Adherence Factors*:

The adhesion to host tissues has been attributed to expression of range fimbrial and non fimbrial adhesins. In common with other members of the Enterobactriaceae .The adhesive properties in the Enterobactriaceae are generally mediated by different types of pili. Pili are nonflagellar, filamentous projections on the bacterial surface.

There are two predominant types in *Klebsiella spp.* Type 1 (common) pili and type 3 pili are the best investigated of the bacterial adhesive are the best investigated of the bacterial mannose sensitive haemagglutination. Type 3 fimbrial also cause haemagglutination of eruthrocyte pre treated with tannin. Additional fimbrial structures (type 6 and kpf 28) and non fimbrial adhesive have been described . Adhesive of 29 k is a plasmd encoded protein that enables strains to adhere to cultured human intestinal cell lines(Podschun and Ullmann, 1998; and Green wood,*et al.*, 2002).

1.2.5.1.3. 2.4. *Haemolysin and Siderophores:*

All strain of *K. pneumoniae* can not produce haemolysin on blood agar (Brook *et al.*, 2004). *K. pneumoniae* expressed a plasmid encoded aerobactin mediated high affinity iron chelating siderophores (Green wood,*et al.*, 2002).*K. pneumoniae* produces two types of siderophores that are capable of competitively taking up iron bound to host proteins. The production of both enterobactin , and aerobactin . In the genus *K . pneumoniae* has been demonstrated. However, enterobactin is synthesized by almost all strains (Podschun, *et al.*, 1992).

1.2.5.1.4 *Pseudomonas aeruginosa:*

P.aeruginosa is belonging to the bacterial family Pseudomonadaceae. It is a Gram-negative rod, non spore forming , usually motile by one or two polar flagella (Green wood,*et al.*, 2002) It is strict aerobic , but it grows in the absence of O₂ if NO₃ is available as a respiratory electron acceptor. *P. aeruginosa* has very simple nutritional requirements, sometimes producing a sweet or grape like or corn taco like odor (Ryan and Ray 2004). Some strains hemolyse blood, *P. aeruginosa* forms mucoid colonies as a result of over production of alginate, an exopolysaccharide. Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees. Most strains of *P. aeruginosa* produce diffusible pigments, typically, the colony and surrounding medium greenish blue due to production of a soluble blephenazin pigment, pyocyanin ,and yellow green fluorescent pigment

pyoverdinin(which act as the major siderophore), additional pigments include pyorubrin (red) and black pigment pyomelanin (Iglewski ,1996).

P. aeruginosa is catalase positive,it differs from members of the Enterobacteriaceae by deriving energy from carbohydrates by a positive oxidase reaction, rather than a fermentative metabolism (ei *P. aeruginosa* is oxidase positive and not fermenter for carbohydrates such as *P. aeruginosa* inability to ferment lactose),one *Pseudomonas* pigment is probably a determinant of virulence for the pathogen.(Green wood,*et al.*, 2002; and Todar,2004)

1.2.5.1.4. 1.*Pathogenesis* :

An opportunistic pathogen of immuno compromised individual. The pathogenesis of *Pseudomonas* infections is multifactorial, as suggested by the number and wide array of virulence determinants possessed by the bacterium (Miyata *et al*, 2003). Multiple and diverse determinants of virulence are expected in the wide range of diseases caused, which include septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis, dermatitis, and osteochondritis bone and joint infections, gastrointestinal infections, eyes infections, Central Nervous System infections, and Skin and soft tissue infections, including wound infections, pyoderma and dermatitis(Rahme *et al*, 1995; and Prithivirai *et al* ,2005).

P. aeruginosa was the most common aerobic organism isolated from chronic OM , it was recovered in 46.6%,38.6%, and 60.7%from all cases of chronic OM (ALKhafaji, 1993; Aubaid, 1997; and Brook ,1998). Most *Pseudomonas* infections are both invasive and toxinogenic. The ultimate *Pseudomonas* infection may be seen as composed of three distinct

stages:(1) bacterial attachment and colonization;(2) local invasion; (3) disseminated systemic disease. However, the disease process may stop at any stage. *P. aeruginosa* is primarily a nosocomial pathogen(Todar, 2004).

1.2.5.1.4. 2 .Virulence Factors :

Most *Pseudomonas* infections are both invasive and toxinogenic.The ultimate infection may be seen as composed of three distinct stages : (1) bacterial attachment and colonization (2) local invasion (3) disseminated systemic disease (Kenneth, 2002). *Ps. aeruginosa* has a series of virulence associated antigens such as fimbriae, slime layer, proteases, cytotoxins (Todar, 2004). These antigen and their specific host responses will be mentioned with ear mucosal response to infections

1.2.5.1.4. 2.1 Capsule:

The mucoid exopolysaccharide produced by *P. aeruginosa* is a repeating polymer of mannuronic and glucuronic acid referred to as alginate. The ability of *P. aeruginosa* to invade tissues depends upon its resistance to phagocytosis and to the host immune defense, and the extracellular enzymes and toxin that break down physical barriers and contribute to bacterial invasion. (Prithiviraj *et al* .,2005). Biofilm mucoid strains of *P. aeruginosa* are also less susceptible to antibiotics.The ability of *P.aeruginosa* to invade tissues depends upon effectively protects cells from opsonization by antibodies, complement deposition, and phagocyte engulfment. (Todar, 2004 ; and Walker *et al* .,2004).

1.2.5.1.4. 2.3 .*Extracellular Proteases* :

The ability of *P. aeruginosa* (as bacterial protease) to invade tissues depends upon production of extracellular enzymes and toxins that break down physical barriers and damage host cells, as well as resistance to phagocytosis and the host immune defenses (Todar, 2004) . Two extracellular proteases have been associated with virulence that exert their activity at the invasive stage: elastase and alkaline protease. Elastase has several activities that relate to virulence. The alkaline protease cleaves immunoglobulin. It also lyses fibronectin to expose receptors for bacterial attachment on the mucosa of the lung. Elastase disrupts the respiratory epithelium and interferes with ciliary function. Alkaline protease interferes with fibrin formation and will lyse fibrin. Together, elastase and alkaline protease destroy the ground substance of the cornea and other supporting structures composed of fibrin and elastin. Elastase and alkaline protease together are also reported to cause the inactivation of gamma Interferon (IFN) and Tumor Necrosis Factor (TNF) (Rahme *et al.*,1997).

1.2.5.1.4. 2.4 .*Adherence Factors* :

Pili (fimbriae)extend from cell surface and promot attachment to host the epithelial cells of the upper respiratory tract and, by inference, to other epithelial cells as well. These adhesins appear to bind to specific galactose or mannose or sialic acid receptors on epithelial cells. Colonization of the respiratory tract by *Pseudomonas* requires fimbrial adherence and may be aided by production of a protease enzyme that degrades fibronectin in order to expose the underlying fimbrial receptors on the epithelial cell surface. Tissue injury may also play a role in colonization of the

respiratory tract since *P. aeruginosa* will adhere to tracheal epithelial cells of mice infected with Influenza virus but not to normal tracheal epithelium. This has been called opportunistic adherence, and it may be an important step in *Pseudomonas* keratitis and urinary tract infections, as well as infections of the respiratory tract. Besides pili and the mucoid polysaccharide, there are possibly two other cell surface adhesins utilized by *Pseudomonas* to colonize the respiratory epithelium or mucin. Also, it is likely that surface-bound exoenzyme S could serve as an adhesin for glycolipids on respiratory cells. (Todar, 2004; and Prithiviraj, *et al*.,2005).

1.2.5.1.4. 4.2 .Other Virulence Factors:

I. *Siderophores* :*Pseudomonas* produce pigments such as pyoverdinin pigment which acts as the major siderophore production fluorescent *in vivo* allows *P. aeruginosa* to compete with mammalian iron binding proteins such as transferrin , siderophore that is produced under low-iron conditions to sequester iron from the environment for growth of the pathogen. No role in virulence is known for the fluorescent pigments. (Todar, 2004 ; Greenwood, *et al.*, 2002 and Sorensen, *et al.* 1983) found that substances with low molecular weight like pyocyanin inhibit lymphocyte proliferation. Kharazmi, *et al.* (1984) found that the effect of alkaline protease and elastase stopped chemotaxis in agarose.

II *P. aeruginosa* produces three other soluble proteins involved in invasion: a cytotoxin (mw 25 kDa) and two hemolysins. The cytotoxin is a pore-forming protein. It was originally named leukocidin because of its effect on neutrophils, but it appears to be cytotoxic for most eukaryotic cells. Of the two hemolysins, one is a phospholipase (heat labile) and the

other is a lecithinase (heat stable glycolipid). They appear to act synergistically to break down lipids and lecithin. The cytotoxin and hemolysins contribute to invasion through their cytotoxic effects on eukaryotic cells .

III. *P. aeruginosa* produces two extracellular protein toxins, Exoenzyme S and Exotoxin A.

1.2.5.1.5 Moraxella catarrhalis:

The genus *Moraxella* is a member of the family Nisseriaceae *M. catarrhalis* was previously named *Branhamella catarrhalis* and before that *Nisseria catarrhalis*(Brook *et al.*, 2004).The organism is characterized as gram negative, aerobic, oxidase positive,catalase positive, diplococci. They are commensals of mucosal surfaces of upper respiratory tract and occasionally give rise to opportunistic infection. *M. catarrhalis* can be differentiated from the other *Nisseriae* by its lack of carbohydrates fermentation and by production of DNA ase. It produces butyrate esterase, which forms the basis for rapid flourometric tests for identification (Green wood,*et al.*, 2002).

1.2.5.1.5.1.Pathogenesis :

M. catarrhalis colonizes primarily the upper respiratory tract. Nasopharyngeal colonization with *M. catarrhalis* is common throughout infancy (Faden *et al.*,1994) *M. catarrhalis* has generated investigative interest because of its importance as a human pathogen in certain clinical settings. It is predominantly a mucosal pathogen. A high rate of colonization with *M.catarrhalis* is associated with an increased incidence

of OM in children, studies from several centers in the 1990s have established that *M. catarrhalis* is the third most common causes of OM (Klingman *et al.*,1995) *M. catarrhalis* causes sinusitis in adult and children and causes lower respiratory tract infection , particularly in the setting of chronic bronchitis and chronic obstructive pulmonary disease(COPD) infection. Invasive infections rarely caused by *M. catarrhalis* include meningitis, endocarditis, bacteremia, wound infection and conjunctivitis (Wallace and Oldfield,1990; Alaeus and Stiernstedt, 1991;Cimolai and Adderley, 1989).Librations of endotoxin, histamine and chemotactically active factors can be considered the major pathogenicity factors of *M.catarrhalis*. The surface of the bacteria contains an outer membrane protein (Karalus and Compagnari, 2000). The pathogen can protect it self by binding of the C1a sub component of the complement system followed by subsequent formation of a functionally inactive complex with C1 and on the other hand, by inactivation of the terminal lytic complement complex by means of specific protein on the surface of the outer cell wall (Murphy,1996 and Cullman,1997).

1.2.5.1.5. 2 .Virulence Factors

1.2.5.1.5. 2.1 .Capsule :

A capsule is a loose, relatively unstructured network of polymers that covers the surface of an organism.The capsular polysaccharides are essential virulence factors (Rubens, *et al.*, 1987). They inhibit phagocytosis and causes complement inactivation in the absence of specific antibody (Bliss and Silver, 1996). Some pyogenic intracellular cocci have the capacity to kill phagocytosis (Gray *et al.*,1999)

Mellenkvist *et al.*, (2003) the *M. catarrhalis* strains donot have capsule. Ahmed *et al.*,(1991) the *M.catarrhalis* strain contain capsular polysaccharide but this capsule can not be differentiated .

1.2.5.1.5. 5.2 . *Adherence and Colonization Factor:*

The first host barrier for many invading pathogens is usually a mucosal surface, and since epithelial cell turnover is around 48 hours in these environments, the bacterium must attach and replicate sufficiently to avoid being swept away. Therefore, many have evolved motile or attachment elements like flagella and pilli to cross the barrier and invade (Sauer, *et.al.*, 2000). Simple attachment is mediated through a receptor on the host cell surface, and an adhesion on the bacterial one. (Mouricout, 1997).

The fimbriae mediated haemagglutination could be resistant or sensitive to blocking by D-mannose (Duguid, *et al.*, 1996).

The most strains of *M. catarrhalis* have numerous pili extensions from cell surface. Piliated strains of *M. catarrhalis* are better abliliy to attach to human mucosal surfaces than non piliated organisms.

1.2.5.1.5. 2.3 *Siderophores & Hemolysin:*

Like other organisms, bacteria require iron as a co-factor for redox-dependent enzymes. Iron is an oxidant as well as a nutrient for invading microbial and neoplastic cells. Siderophores are chelating compounds, which are small (MW 500-1000) lignand that are specific for ferric iron and thus they supply iron to the bacterial cell (Griffiths, *et.al.*, 1987).

The iron is then released from the ferrisiderophore complexes for incorporation into cellular protein. (Drechsel, *et al.*, 1997).

Many studies (Campaynarri *et al.*, 1992) had mentioned that *M.catarrhalis* have high affinity for use of lactoferrin and transferrin and hemoglobin receptors as a source of iron in the body.

The bacterial hemolysin is one of virulence factors but *M.catarrhalis* strains do not produce the hemolysin (Catlin, 1990)

It is known that the bacteria which is able to produce hemolysin have no ability to produce siderophore and vice versa (Al-Zaag, 1994).

1.2.5.1.5. 2.4 .Extracellular Protease Production:

Proteases represent a class of enzymes that are involved in essential biological processes like blood clotting, controlled cell death, and tissue differentiation. They catalyse important protolytic steps in tumor invasion or in infection cycle of a number of pathogenic microorganisms and viruses. Proteases assist the hydrolysis of large cellular polypeptides . The extracellular enzymes play a major role in nutrition due to their depolymerizing (Beynom and Bond, 1989).

Numerous proteases are produced by microorganisms depending on the species of the producer or the strain even belonging to the same species. Several proteases are also produced by the same strain under various culture conditions (Rae, *et al.*, 1998).

Most bacteria proteases excreted into infected host exhibit a wide range of pathogenic potentials ranging from pain, edema or even shock to translocation of bacteria from the site of infection into systemic circulation, thus resulting in septicemia (Maeda and Yamamoto, 1996).

Perez *et al.*, (1990) had mentioned that many strain of *M. catarrhalis* can produce amino peptidase.

1.2.1.6. Other bacterial causes OM

1.2.1.6. 1. *Haemophilus influenzae*:

H. influenzae is a small, pleomorphic, gram-negative coccobacilli. It is non-motile, non-spore-forming, fastidious and facultative anaerobe. Some strains of *H. influenzae* possess a polysaccharide capsule, and is found principally in the upper respiratory tract (Hacker, *et al.*, 1997; Brook *et al.*, 2004). *H. influenzae* "Loves heme" more specifically requires a precursor of heme in order to grow. Nutritionally, *H. influenzae* prefers a complex medium and requires preformed growth factors that are present in blood, specifically X factor (i.e. hemin) and V factor (NAD or NADPH). In the laboratory, it is usually grown on chocolate agar which contains both X and V factors (Todar, 2004).

The bacterium grows best at 35 – 37 °C and has an optimal pH of 7.6. *H. influenzae* generally grows in the laboratory under aerobic conditions or under slight CO₂, although it is capable of glycolytic growth and of respiratory growth using nitrate as a final electron acceptor (Moxon and Murphy, 2000). Strains are classified as either capsulated (typeable) strains (based on the antigenic structure of the capsular polysaccharide), and uncapsulated (nontypeable) strains. There are six structurally and antigenically distinct capsular types (serotypes) designated serotypes A to F (Joseph and St Geme, 2002).

Harabuchi *et al.*, (1994) stated that non typeable strains do not produce a capsule and are most commonly encountered as normal inhabitants of the URT, non capsulated strains usually cause localized infection such as sinusitis, otitis media, acute and chronic exacerbations of lower respiratory tract infection, and acute and chronic conjunctivitis

(Nizet *et al.*, 1992). The virulence factors that may be involved in pathogenicity include : (1)Fimbriae,which assist attachment to epithelial cells.(2) IgA proteases, which are also involved colonization. (3) Outer membrane components , including outer membrane proteins and lipopolysaccharide , which may contribute to invasion of several stages (Green wood *et al.*, 2002).

1.2.1.6.2.*Proteus* spp:

It is a small gram negative ,oxidase negative ,urease positive grow on potassium cyanide medium (kcn), and not ferment lactose on MacConkey agar, *Proteus* move very actively by means of peritrichous flagella, resulting in swarming on solid media (*Proteus* produce swarmer cells which are elongated forms created when cells fail to septate or divide, these cells, which are profusely covered with flagella, act in concert to produce swarming motility on solid media) (Brook *et al.*,2004). Strains of *Proteus mirabilis* cause of urinary tract infection(Green wood *et al.*, 2002)

Proteus spp is common cause of chronic OM (Senior and Sweeny,1984; and Sweeny *et al.*, 1982)

This bacteria have many virulence factors like fimbriae, flagella, outer membrane proteins, capsule antigen, lipopolysaccharide, and IgA proteases, which attribute to the pathogenicity of this bacteria (O' Hara *et al.*, 2000;Green wood *et al.*, 2002)

1.2.1.6.3.*Escherichia coli*:

One of most important Enterobacteriaceae species is gram negative rods ,usually motile, produce polysaccharide capsule , positive tests for indol,

lysine decarboxylase and mannitol fermentation, and produces gas from glucose. Typical morphology with an iridescent sheen on differential media such as EMB agar, and they grow on non-selective media, most strains ferment lactose producing large red colonies on MacConkey agar and this bacteria is predominant among aerobic commensal bacteria in healthy human intestine (Smith and Scotland 1993; Brook *et al.*, 2004).

E. coli has many virulence factors that enable it to cause disease and the most important virulence factors are polysaccharide of somatic antigen O and capsular K antigen which protect the organisms from bacteriocidal effect of complement and phagocytosis in the absence of antibody (Chart, 1998). Many strains express haemolysin S and in general strains of *E. coli* isolated from extra-intestinal infection are more haemolytic than those isolated from the faeces of healthy humans (Chart and Tenkin, 1999). *E. coli* causes UTI, chronic OM and sinusitis (Adhhan, *et al.*, 2005).

1.2.1.6.4. *Staphylococcus epidermidis*:

It is gram positive, catalase positive, oxidase negative, coagulase negative, *S. epidermidis* has a relatively small white colony, this type of *Staphylococci* constitutes a major component of the normal flora of humans, causing nosocomial infection, *S. epidermidis* is an opportunistic pathogen that causes infection in debilitated or compromised patients such as chronic OM (Kloss and Bannerman, 1994; Brook *et al.*, 2004). Virulence factors of coagulase negative *Staphylococci* follow initial colonization, a copious amount of extracellular polysaccharide or slime which many correlate with pathogenicity and bacterial adherence (Heikens *et al.*, 2005).

1.2.1.6.5 . *Streptococcus pyogenes* :

It is gram positive , catalase negative , oxidase negative, facultative anaerobic bacteria, form spherical , characteristically form chains during growth (Brook *et al.*,2004).

Beta hemolytic , bacitracin susceptible contain A antigen, It is the main pathogen which causes a wide range of suppurative infections in the respiratory tract and skin life threatening soft tissue infections (Kirby and Tenover,1995). Strain of *S. pyogenes* express a large arsenal of virulence factors and hence their pathogenicity and the clinical signs that they induce are very diverse (Boyle, 1995). The adhesion by interaction with host fibronectin a matrix protein on prokaryotic cells , is considered the principal mechanism by which *S. pyogenes* bind to epithelial cells of respiratory tract and skin (Kilian,2002).

1.2.1.6.6. *Anaerobic Bacteria*:

Although anaerobic organisms have infrequently isolated from chronic OM because of the techniques employed in the isolation of such as organisms (Erkan *et al.*,1994). The foul smell of chronic ear discharge and the high infrequency of anaerobic bacteria in otogenic intracranial infections give a good support for anaerobic bacteria as an important component of the bacterial flora in chronic OM (Al-Mahmood and Al-Karagoli,1999). Anaerobic bacteria represented by anaerobic gram positive cocci (50%) and *Bacteroides* sp (33.3%) was presented in children younger than four years old .In Saudi Arabia , anaerobic bacteria presented in (0.9%) which included *Bacteroides* sp only (Ibekwe *et al.*,1997). In Iraq, *peptococci* (30%) and *Bacteroides fragilis* (11.8%) was

the most anaerobic isolates which presented in mixed with aerobic bacteria (ALKhfaji, 1993) .In recent study ,the pre reduced michrome wire swab in staurts medium for isolation of anaerobic bacteria in a large numbers than by conventional ear swab (ALMahmood and ALKaragoli,1999) .*Peptostreptococcus sp* and *Fusobacterium* were also considered the most anaerobic bacteria causing COM in various frequencies(Brook *et al.*, 1998).

1.2.1.6.7. *Mycoplasma pneumoniae* :

M. pneumoniae donot appear to play a significant role in acut OM , some patients with lower resipartory tract disease due to *M.pneumoniae* may have concomitant OM (Klein, *et al.*,2000).

1.2.1.6.8. *Clamydia trachomatis*

C. trachomatis is associated with acute resipartory infection in infant younger than 6 months and is cause of acute infection of the ME in this age group (Klein,*et al.*,2000).

1.2.5.2 *Fungal infection* :

Fungus is more secondary invader like other non pathogenic microorganisms, and it was invariably isolated in chronic OM, especially from hot, humid regions of the world (Dincer *et al.*, 1992).

In otolaryngology fungal infection often regarded as colonization rather than invasion. In Saudi Arabia it was reported Ibekwe *et al.*,(1997) that fungi accounted for 25% from all isolates in which *Aspergillus sp* and

Candida sp presented in 17.1% and 8.1% respectively. Fungal pathogens were also reported as CSOM causes in Basrah city (Yassin,1990) and in Baghdad (ALKhfaji, 1993) .

1.2.5.3 Viral infection:

Respiratory viruses have been implicated as causative agents in acute OM. The URT viral infection have been shown to precede as many as 50% of episodes of acute OM. (Arola, *et al.*, 1988). Viruses have been isolated in nearly 75% of ME fluids in children with acute OM. (Pitkaranta *et al.*, 1998; Heikkinen, 1999). The most common viruses, *rhinoviruses*, *mumpsviruses*, *enteroviruses*, *adenoviruses*, and *para influenza viruses*. Viruses have been implicated as a cause of treatment failure in cases of acute OM. (Chonmaitree *et al.*, 1992 ; Buchman *et al.*, 2002).

1.2.6 Treatment of Otitis Media :

Treatment of OM may include medical , surgical or both, depending on the patient condition, severity of the disease and type of etiological agents (Kenna, 1994).

Medical treatment of OM is an important step in achieving a drug ear (Indudharan *et al.*, 1999), and it includes either aural toilet or antimicrobial agents, and the latter (antimicrobial agents) may be used either topically or systemically or by both routes. Topical treatment may include antiseptics or antimicrobial agents which have been extensively used in the treatment of adults (Diner *et al.*, 1992). Two categories of antimicrobial agents are widely used ; aminoglycosides and β lactam antibiotics both in combination with or without steroid antifungal (Shenio , 1987).

Many studies revealed that gentamycin (Indudharan *et al.*,1999), parenteral cefotazidime (Dangan *et al.*,1992); topical phosphomycin (Claes *et al.*,1992); 0.3% of ofloxacin ear drops (Yuen *et al.*, 1996)and combined tobromycin – dexamethasone ear drops (Alper *et al.*, 2000)were the most effective antibiotics in initial treatment of chronic OM and in addition to that ,topical use of ciprofloxacin gave very good results , especially for pseudomonas infection and methicillin resistant *Staphylococcus aureus* (MRSA)(Brownlee *et al.*, 1992;and Epissito *et al.* , 1992). Some studies showed that topical use of a mixture containing neomycin and polymyxin B may contribute to sensorineural hearing loss in patients with chronic OM (Podoshin *et al.* , 1989).Recent studies showed that the used systemic antibiotic appeared to be an effective treatment of COM against a wide range of gram negative and gram positive organisms and 95% of patients revealed either improvements or cure (Legent *et al.*,1994). Amoxicillin, augmentin and second generation of cephalosporins were the most common systemic antibiotic used for OM treatment(Ibekwe *et al.*, 1997). Allergy therapy may be used to reduce the morbidity and health care costs associated with OM in the presence of allergic rhinitis(Fireman, 1997).

1.2.7 Immunology

The middle ear is the site of a secretory immune system similar to those of other areas of the respiratory tract. Local and systemic immune responses occur in patients with acute or chronic otitis media with effusion. In the middle ear , immunologically active antigen interacts with immunocompetent cells in the lamina propria to produce a local immune response . The middle ear effusion that results from acute or chronic

infection contains all the major classes of immunoglobulins , competent cells, immune complex of antigen and antibody, and various chemical mediators of inflammation. The role of these substances in the course of OM is uncertain. The immune response against various antigens may prevent subsequent infection, assist in clearance of fluid during the acute episode, or contribute to the accumulation and persistence of fluid in the middle ear cavity. (Klein, *et al.*, 2000).

The mucous layer of the upper respiratory tract contains several substances having antibacterial effect like lactoferrin, lysozyme, fibronectin, lactoperoxidase (Stenfors, 1999).

Another mechanism against infection in the upper respiratory tract is the production of SIgA antibody by mucosal lining and lymphoid ring e.g. the adenoid. SIgA in nasopharyngeal secretions inhibits bacterial adherence and reduced nasopharyngeal bacterial colonization, both important factors in the protection against otitis media (Lim and Mogi, 1994),

The fourth bacteria *S. pneumoniae* , non typable *H. influenza* , and *M. catarrhalis* and *S. aureus* cause 60% of the infections .

Furthermore, it has been shown that the act of immunization , by parenteral injection with certain capsular polysaccharides, can prevent the development of OM in animal models and in children (Robbins *et al.*, 1988). Inflammatory cytokines which are host glycoproteins produced by macrophages and other cells mediate the inflammatory reaction and regulate the immune response within the ME while it is likely that nasopharyngeal pathogenic organisms are related to the development of OM with effusion, it is possible that the thick mucoid secretions developing within the ME (Stenfors and Räsänen, 1992). Clinical and experimental observations suggest that immunoglobulin may prevent OM in children ,

performed experiments in chinchillas to test the hypothesis that human antibacterial polysaccharides immunoglobulin (BPIG) might prevent OM caused by *S. pneumoniae*, at levels of anticapsular immunoglobulin G similar to those of human adults, BPIG given systemically prevent pneumococcal OM and disseminated infection in chinchillas. BPIG may be of value in preventing human bacterial infection and may also inhibit development of antibody if it affects local infection or colonization. Specific IgG antibody may provide an important antibacterial defense of mucosal surfaces of the respiratory tract. Recent studies have shown that chinchillas are protected from experimental ear infection with non-typable strains of *H. influenzae* after either recovery from homologous strain infection or by systemic administration of immune chinchilla serum. *S. pneumoniae* is the most important bacterial agent of OM; new approaches to prevention of pneumococcal OM are of particular interest (Shurin, *et al.*, 1988).

1.2.8 . Detection of uronic acid

Uronic acid is a sugar acid with both a carbonyl and carboxylic acid (on the carbon that is not part of the ring) (Wikimedia, 2007). The name colanic acid was applied by Goebel (1963) to an exopolysaccharide slime synthesized by a mucoid strain. The acid production controlled by a regulator gene, such as AlgI-AlgJ-AlgF and AlgK may form a complex in the membrane that is the reaction center for O-acetylation of alginate of *P. aeruginosa* (Gimmestad, *et al.*, 2003). The exopolysaccharide slime colanic acid has been isolated from representative strains of *Enterobacteriaceae*. Analysis showed that each polymer contained

glucose, galactose, fructose and glucuronic acid, together with acetate and pyruvate. The names of uronic acid are based on their parent sugars, in *S. pneumoniae*, *K. pneumoniae*, *S. aureus*, called glucuronic acid while in *P. aeruginosa* called mannuronic acid, the important role for the uronic acid component in their antigenicity. The K antigens were found to be acidic heteropolysaccharides consisting of seven different monosaccharide constituents. Mostly glucuronic acid represents the acidic component. Galacturonic acid was found to be present only in six antigens. The capsular polysaccharides contain at least two other sugars in addition. Various combinations of monosaccharides were observed. Some of these have the same qualitative sugar composition but differ with respect to their serological specificity (Ratto, 2006).

Materials and Methods

2. 1 Patients:

One hundred discharge samples were collected from patients suffering otitis media (O M) the period from November 2006 through March 2007 at Hilla teaching hospital (ENT).

All patients subjected to this study were distributed according to their ages, sexes, duration of the disease, and using of Antibiotics

2-2 Materials:-

2.2.1 Laboratory Instruments

No.	Instruments	Company
1	Sensitive Electronic Balance	A and D, Japan.
2	Autoclave	Stermite, Japan.
3	Incubator	Memmert, Germany.
4	Distillator	GFL- Germany.
5	Centrifuge	Hermle, Japan.
6	Oven	Memmert, Germany.
7	Refrigerator	Concord, Italy.
8	Milipore Filter	Satorius membrane filter Gm bH, W. Germany.
9	Light Microscope	Olympus, Japan.
10	Micropipette	Oxford, USA.
11	pH Meter	Hoeleze and Cheluis, KG, Germany.
12	Water Bath	Memmert, Germany

2.2.2 Chemical and Biological Material

Name of material	Company
A- Chemical Materials	
K ₂ HPO ₄ , NaCl, MgSO ₄ , HCL, CaCl ₂ , KOH	Merk-Darmstadt.
α-nepththol amine, Tetramethyl-p-paraphylene diamine dihydrochloride, trichloroacetic acid, Amyl alcohol, Methyl red, Chloroform, gelatin, ,galactouronic acid, peptone	B.D.H
H ₂ O ₂ , Glucose, dipyridyle, 99% alcohol. carbazole	Fluka chemika- Switzerland
B- Culture media	
Blood agar base, MacConkey agar , Agar-agar, Muller- Hinton agar, Pepton water medium, Nutrient agar media, Nutrient broth,Cimmon citrat,Kligler iron agar	Hi medium

2-2-3 Diagnostic Disks:-

1. Bacitracin (10 IU) (Himedia, India)
2. Optochin (ethyl hydrocuprein hydrochloride 5µg) (Himedia, India)

2-3 Methods:-

2-3-1 Collection of Specimens:-

The proper specimens collected for bacteriological analysis are described below. Those specimens were collected under the help of advisory to avoid any possible contamination. The samples are generally collected from patients with otitis media. The swabs are inserted in to the middle ear and rotated there before with drawing it and with down without contamination from the external ear, so that exudate is collected from middle ear. Swab for culture should be placed in tubes containing normal saline to maintain the swab moist until taken to laboratory. Each specimen was immediately inoculated on the blood agar plates, nutrient agar, chocolate agar plates and MacConkey's plates. All plates were incubated aerobically and anaerobically by candle jar at 37 C° for 24-48 hrs. and the ear discharge were collected to uronic acid detection. The swabs were collected from (10) healthy as a control group (these without any clinical sign and symptoms of otitis media).

2-3-2 Preparation of The Reagent and Solutions:-

2-3-2-1 Oxidase Preparation:-

This reagent was prepared by dissolving 1 gm of (tetramethyl-paraphenylene-diamine-dihydrochloride) in 100 ml of D.W and immediately used for identification the bacteria (Baron, *et al.*, 1994).

2-3-2-2 Catalase Preparation :-

This reagent was prepared in (3%) using H₂O₂ dilute by D.W and stored in a dark container used for identification the bacteria (Baron, *et al.*, 1994).

2-3-2-3 Methyl Red Reagent:-

Methyl Red Reagent was prepared by dissolving 0.1 gm of methyl red in 300 ml of 95% ethanol and then the volume was completed to 500 ml by D.W(Macfaddin, 2000).It was used to detect the complete glucose hydrolysis .

2-3-2-4 Vogus-Proskaur Reagent:-

It is composed of two solutions as below:-

- a- α -Naphthol-reagent was dissolved in 100 ml of 99% ethanol.
- b- Potassium Hydroxide (KOH) solution: - 40 gm of KOH was dissolved in 100 ml of D.W. and it was used to detect the partial glucose hydrolysis (Collee *et al.*, 1996).

2-3-2-5 Kovac's Reagent:-

It was prepared by dissolving 5 gm of P-dimethylamine benzylaldehyde in 75 of amyle-alcohole and was added 35 ml of concentrate HCl acid. It was used to detect the Indol production (Baron, *et al.*, 1994).

2-3-2-6 Phosphate buffer solution:

Eighty gm of NaCl, 0.34 gm of KH_2PO_4 , and 1.12 gm of K_2HPO_4 are all dissolved in 1000 ml of D.W. The pH is 7.3, then the solution is sterilized in autoclave ,It was used to detect the Haemagglutination Test (Baron, *et al.*, 1994).

2-3-3 preparation of The Culture Media:-

The general culture media described below were prepared by the routine methods and used in appropriate experiments:-

2-3-3-1 Blood Agar Medium:

Blood agar medium has been prepared according to Macfaddin (2000) by dissolving 40 gm blood agar base in 1000 ml D.W. and autoclaved at 121C° for 15 min, then cold to 50 C° and 5% of human blood was added. This medium was used to cultivate bacterial strain and to determine their ability to blood haemolysis.

2-3-3-2 Chocolate Agar Medium:

Chocolate agar medium has been prepared by dissolving 40 gm of blood agar base in 1000 ml D.W. and sterilized by autoclaving. Then 8% of human blood was added to the medium after cooling to 80 C°. This medium was especially used for isolation and cultivation of bacteria that need 5-10% CO₂ tension (Baron, *et al.*, 1994).

2-3-3-3 MacConkey Agar Medium:

MacConkey agar medium has been prepared according to the method recommended by the manufacturing company and it is used for the primary isolation of most Gram- negative bacteria and differentiation of lactose fermentative from the non lactose fermentative (Collee *et al.*, 1996).

2-3-3-4 Nutrient Agar Medium:

Nutrient agar medium has been prepared according to the manufacturing company. It has been used for general experiment isolate

culture, cultivation and activation of bacterial isolates when it is necessary (Macfaddin, 2000).

2-3-3-5 Mannitol Salt Agar Media:

This media has been used as a selective media for the isolation of Staphylococci and differentiation of *Staphylococcus aureus* (Macfaddin, 2000).

2-3-3-6 Muller- Hinton Agar:

Muller- Hinton agar has been prepared according to the method recommended by (Cruikshank *et al.*, 1975) and it is used in anti-microbial susceptibility testing.

2-3-3-7 Kligler Iron Agar :

Kligler Iron agar has been used for determining glucose and lactose fermentation and possible hydrogen sulfide H₂S production as a first step in the identification of Gram- negative bacilli (Macfaddin, 2000)

2-3-3-8 Pepton Water Media:

Pepton Water Media was prepared and used to detect the ability of bacteria to produce Indole according to the method described by (Baron, *et al.*, 1994).

2-3-3-9 MR-VP Medium:

MR-VP medium has been prepared and used to detect the partial and complete hydrolysis of glucose according to Macfaddin (2000).

2-3-3-10 Simmons' Citrate Medium:

Simmon's Citrate Medium has been used for determining the ability of bacteria to utilize citrate as the sole carbon source (Macfaddin, 2000).

2-3-3-11 M9 Media:

Six gm of Na_2HPO_4 , 3 gm of KH_2PO_4 , 0.5 gm of NaCl , and 1 gm of NH_4Cl ; were dissolved in 950 ml of distill water with 2% agar, and then sterilized by autoclave. After cooling, 2 ml of 1M of MgSO_4 , 10 ml of 20% glucose and 0.1 ml of 1M of CaCl_2 (sterilized separately by filtration) were added, then the volume was completed to 1000 ml. This media was used for the detection of the Siderophores and Extracellular Proteases production (Miniatis, *et al.*, 1982).

2-3-4 Laboratory Diagnosis:

According to the diagnostic procedures recommended by Maccfaddin (2000); Bergy's manual for determinative bacteriology (Holt, *et al.*, 1994); (Collee, *et al.*, 1996); (Baron, *et al.*, 1994), the isolation and identification of gram positive and gram negative bacteria in the otitis media of patients were performed as follows:-

2-3-4-1 Microscopy Examination and Colonial Morphology:

A single colony was taken from each primary positive culture and its identification depended on the morphology properties (Colony size, shape, color and natural of pigments, translucency, edge, and elevation, and texture), and then colonies suspected to be pathogens were selected and investigated by gram-stain to observe a specific shape, color, aggregation and specific intracellular compounds. After staining the bacteria by gram stains, specific biochemical tests were done to reach the final identification.

2-3-5 *Physiological and Biochemical Tests*

2-3-5-1 *Oxidase Test:*

A filter paper circle was placed into a sterile plastic disposable petridish and moistured with several drops of the freshly prepared oxidase reagent, then a small portion of the colony to be tested was removed and rubbed on the filter paper changing in the color to blue or purple within 10 seconds indicated for a positive result (Baron, *et al.*, 1994).

2-3-5-2 *Catalase Test:-*

By streaking the nutrient agar medium with the selected bacterial colonies and incubated at 37 C° for 24 hrs then transfer the growth by the steak and put it on the surface of a clean slide and add a drop of (3% H₂O₂), positive result when the gas bubbles appear (Baron, *et al.*, 1994).

2-3-5-3 *Coagulase Test:*

This enzyme was tested by two methods:-

a. Slide test for bund coagulase (clumping factor): A drop of human plasma was placed on a clean, dry glass slide, a drop of D.W. was placed next to the drop of plasma as a control. By a sterile loop an amount of the isolated colony was emulsified with each drop. When clumping the plasma, bacteria was observed and a smooth homogenous in the control; the result was recorded positively (Baron, *et al.*, 1994).

b. Tube test for free coagulase:

half ml of Human plasma was placed in a glass tube and a visible portion of growth from isolated colonies was emulsified in the plasma by rubbing the material on the slide of the tube while holding the tube at an angle, then the suspension was incubated for 1-4 hrs at 37 C°; the presence of clot that cannot be resuspended by

gentile; shaking was recorded as a positive result. The organism that fails to clot the plasma within 24 hrs is considered as coagulase negative (Baron, *et al.*, 1994).

2-3-5-4 Citrate Utilization Test :-

The surface of simmon's citrate slant medium was inoculated with colony of the tested bacteria and incubated at 37 C° for 1-3 days. Conversion of the indicator's color from green to blue indicates that the organism was able to utilize citrate as a sole carbon source (Cruikshank *et al.*, 1975).

2-3-5-5 Kligler's Iron Agar Test for H₂S Production:-

Only the colonies growing on MacConkey agar were touched by a straight wire and inoculated on the media by stabbing the butt of the tube and streaking the slant. Fermentation was detected by a change in the indicator phenol red to yellow. The PH changes in the butt and the slant of medium were recorded after 18- 24 hrs of incubation of gas formation which is usually visualized as bubbles in the medium caused by the gas formed in the agar. Organisms can produce H₂S form black precipitate in the butt (Baron, *et al.*, 1994).

2-3-5-6 Indol Test :

Tubes containing a peptone water medium were inoculated with the colony of the tested bacteria and incubated at 37 C° for 18 hrs, then several drops of kovac's reagent were added to the broth medium. After shaking, the appearance of the red ring on the surface was regarded as a positive result (Cruikshank *et al.*, 1975).

2-3-5-7 Methyl Red Test:-

The tubes of the (MR-VP broth) were inoculated with the selected bacterial colonies and incubated at 37°C for 24 hrs, then (5 drops) of methyl red reagent were added to it. The appearance and observation of red colour means a positive result and a complete analysis of glucose (MacFaddin, 2000).

2-2-6-8 Vogus Proskaur Test:-

The tubes of (MR-VP broth) were seeded with the specific bacterial culture and were incubated at 37°C for 48 hrs., then we read the result by adding (0.6 ml of α - naphthol reagent) and (0.2 ml of 40% KOH solution); appearance of red colour after 15 min. means positive result due to partial analysis of glucose, which produce acetone or (Acetyl methyl-carbinol) (MacFaddin, 2000).

2-3-5-9 Mannitol Salt Agar Test:-

The differentiation between *Staphylococcus aureus* and other Staphylococci e.g. *Staphylococcus epidermidis* was done by sub culturing selected colonies on mannitol salt agar for 24 hrs at 37 C°. Colonies surrounded by a yellow halo indicating mannitol fermentation and isolates colony, is *Staphylococcus aureus* (MacFaddin, 2000).

2-3-5-10 Optochin Susceptibility Test:-

A half of 8% human blood agar plate was streaked with an inoculum from a pure isolates of the organism to be tested , then an optochin disc was placed in the center of the inoculum and incubated for 24 hrs at 37 C° in a candle jar , then observation of zones of growth inhibition greater than 14 mm surrounding the disc was considered positive and it was

presumptive indication of *Streptococcus pneumoniae* (Baron, et al., 1994).

2-3-5-11 Bacitracin sensitivity test:

The blood agar was streaked with bacterial culture and the bacitracin disk put in the center of the petri dish. The diameter of inhibition zone was equal to or more than 12 mm, indicating a positive result. This test was used to diagnosis of *S. pyogenes* (MacFaddin, 2000).

2-3-5-12 Motility test

This test was studied as mentioned in MacFaddin (2000).

2-3-6 Virulence Factors Tests

2-3-6-1 Capsule Stain Test (Hiss's Method):

A thin smear of organism is prepared on the slide and allow to air dry. Treat this freshly prepared smear with hot 1% crystal violet for about four minutes.

The smear is washed with 20% solution of copper sulfate and allowed to dry in air, and then it is examined under microscope, capsule appear as a light blue and the body of the bacteria stain a deep purple (Cruickshank, et al., 1975).

2-3-6-2. Blood Hemolysis Production Test :

Blood agar medium was streaked with a pure culture of bacterial isolate to be tested and incubated at 37 C° for 24-48 hrs. The appearance of a clear zone surrounding the colony is an indicator of β - hemolysis while the greenish zone is an indicator of α - hemolysis (Cowan, 1985).

2-3-6-3 Siderophores Production Test:

M9 Media was prepared and then supplemented with 2% agar. After sterilization in autoclave and cooling to 50C°, 0.25 mg/L glucose

(sterilized by filtration) and 200 $\mu\text{mol/L}$ of dipyridyl were added. Then the organisms were inoculated into this media and it was incubated for 24 hours at 37°C . The results were seen when the growth of organism was present or not (Nassif, *et al.*, 1989).

2-3-6-4 Extracellular Protease Production Test:

This method was carried out by using M9 media supplemented with 2% agar. After sterilization in autoclave and cooling at 50°C , 0.25 mg/L glucose (sterilized by filtration) was added, and then, the media was supported by 1% gelatin. After the inoculation of this media with bacterial strain and incubation for (24 - 48) hours at 37°C , 3 ml of Trichloroacetic acid (5%) was added to precipitate the protein. The positive result was read by observing a transparent area around the colony (Piret, *et al.*, 1983).

2-3-6-5 Haemagglutination Test (HA):

It was performed to show the ability of bacterial group to produce colonization factors antigen. RBC suspension was prepared from the human blood (group A). Human blood washed with phosphate buffer saline (repeated 3 times), 3% suspension from RBC (V/V) was then prepared and at the same time, blood agar was prepared, then it was inoculated with bacteria and incubated at 37°C for 24 hours, after that, place 1 drop of human red blood cells with loopful onto a clean slide, to this drop added 1 drop of bacterial culture by using a flamed loop and bacterial culture were mixed with human red blood cells on clean slide. The blood agglutination with bacteria was detected in room temperature during (1-5) minutes. Agglutinated red blood cells in suspension (positive reaction) had a

clumped appearance distinct from non-agglutinated red blood cells (negative reaction) (Francis, *et al.*, 2002).

2-3-7 Antimicrobial Sensitivity Test (Kirby-Bauer Method) :-

I. It was performed by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from 5 isolated colonies grown on blood agar plate to 5 ml of Nutrient broth. This culture was then incubated for 2 hours to produce bacterial suspension of moderate turbidity. A sterile swab was used to obtain an inoculum from the standardized culture. This inoculum then was streaked on a Muller-Hinton plate.

II. The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator.

III. Incubation was usually overnight with an optimal time of 14 hours at 37C°. Antibiotic inhibition zones were measured using a caliper. Zone size was compared to standard zones to determine the susceptibility or resistance of the organism to each antibiotic (MacFaddin, 2000). Antibiotic disc supplied from Oxoid Company can be disc potency by µg/ml concentration as followed:-

CLR*	AZM*	SXT*	CTX*	CIP*	Ery*	Amx*	DO*
µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
15	15	25	30	5	15	10	30

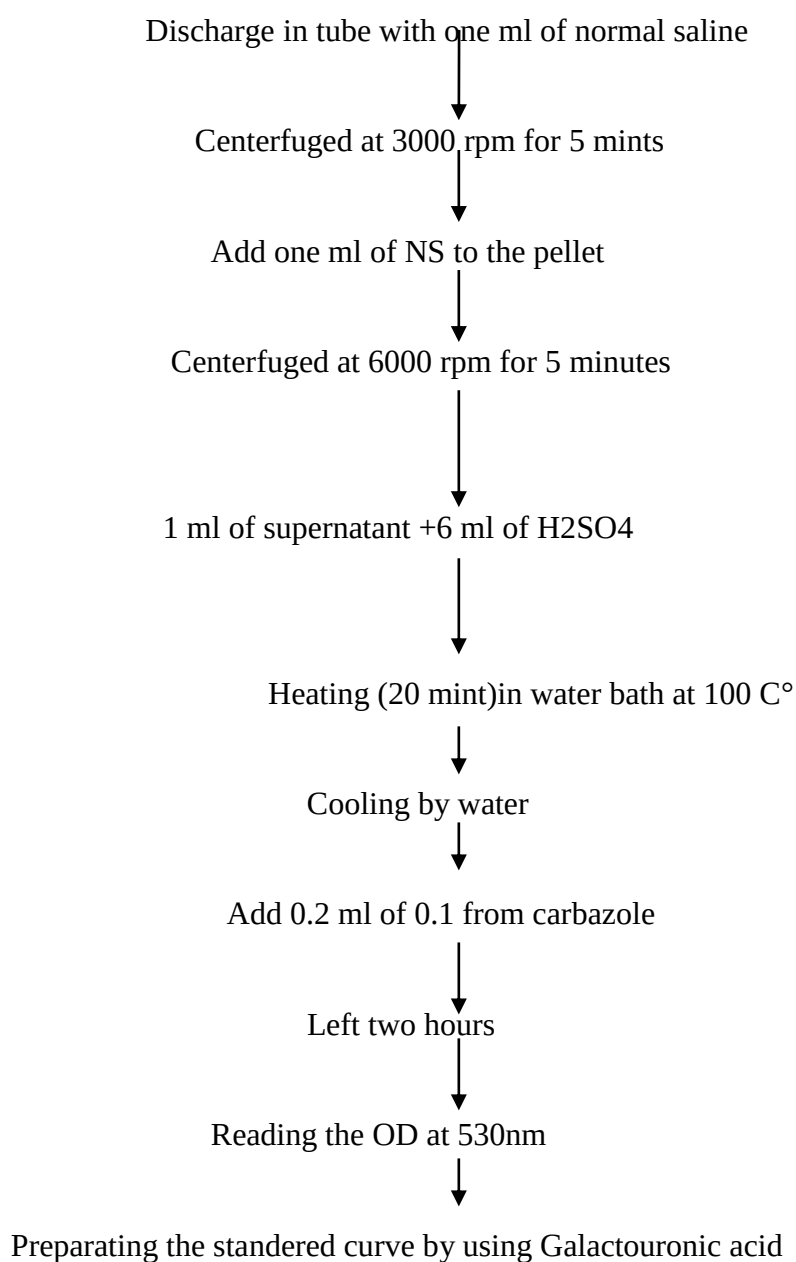
*DO:Doxycycline,ER:Erythromycin.CIP:Ciprofloxacin,AMX:Amoxillin,AZM:Azi
thromycin

CLR:Clarithromycin , SXT:Trimetheprime- sulfamethoxazole CTX:Cefotaxime,

2-3-8- Extraction the polysaccharide and detection from colonic acid :

The extraction the polysaccharide using by {methods of Enzymology part III}, Select 22 samples (mucos discharge in otitis media), the steps of methods shown in chart(1), and preparation the concentration of Galactouronic acid(10,20,40, 50,60,80,100 mircogram /1ml). as suggested by our supervisor(Dr Mohommed Sabri,2007).

Scheme(1)show stages of extraction the polysaccharide in discharge of otitis media



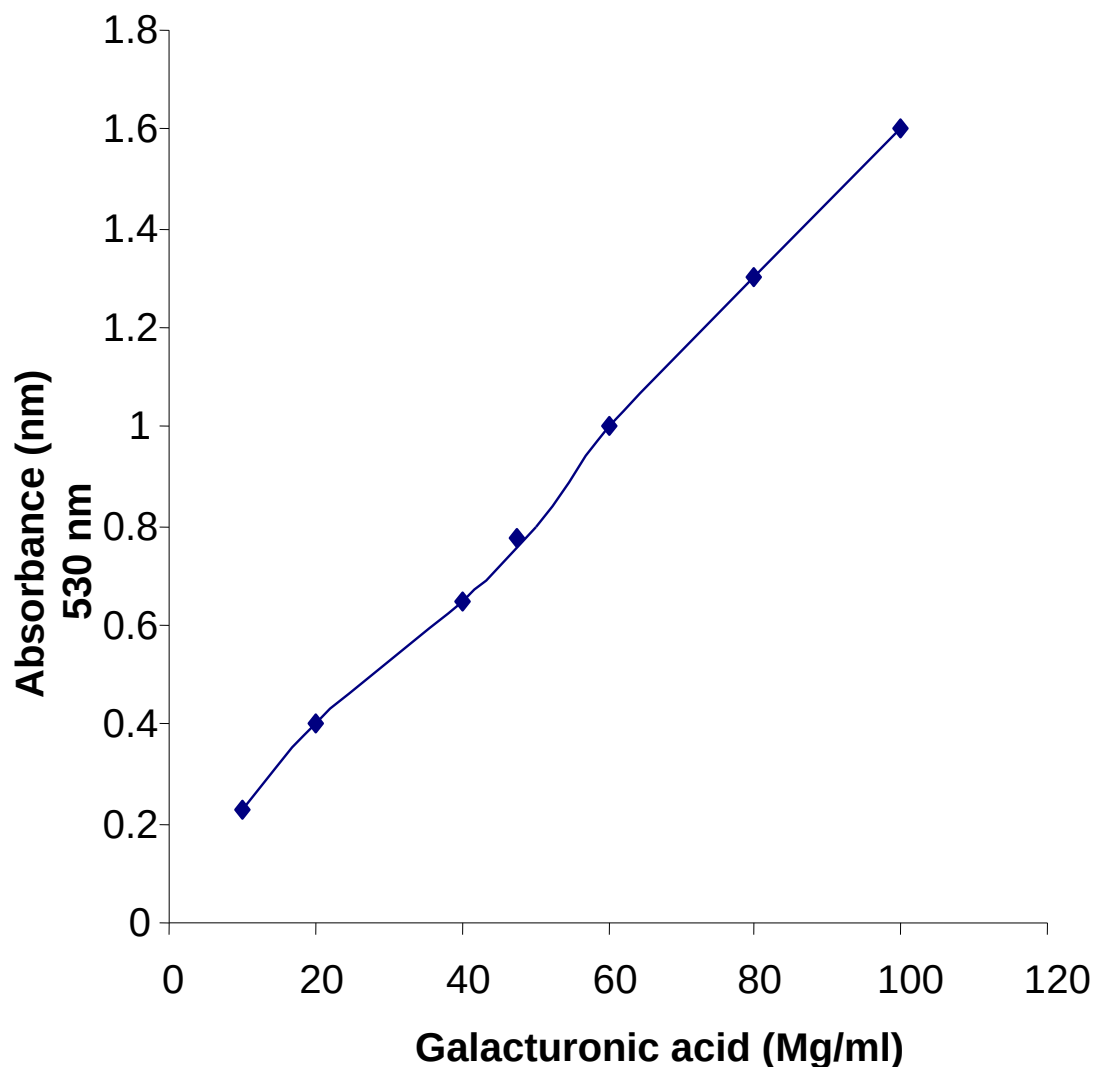


Figure (2-1)The standered curve for producing colonic acid by using galactauronic acid

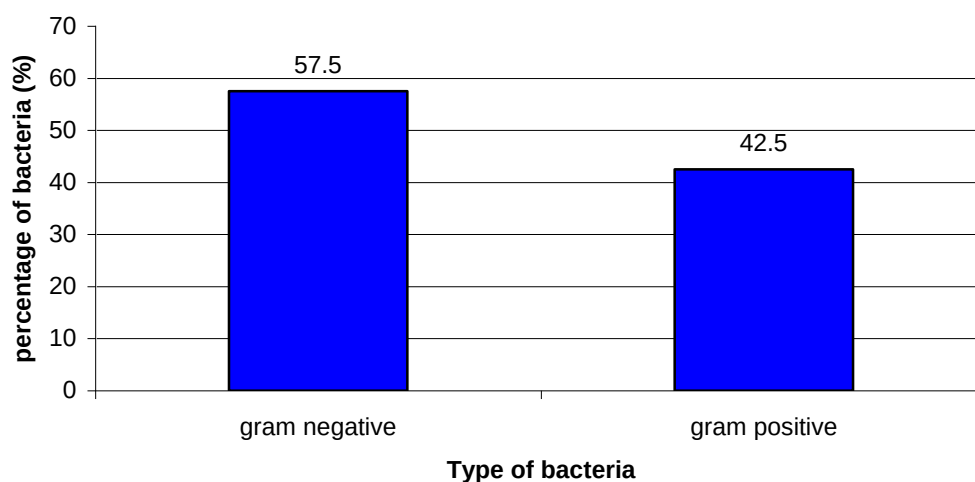
2-3-9- Statistical Analysis:

Differences between males and females were compared by X^2 tests under confidence level of 0.95; the P value ≤ 0.05 ; was considered a no significant difference

3.1. *Bacterial Isolates from otitis media:*

A total of 100 otitis media swabs were subjected for culturing on different types of culture media, the results revealed that 95 samples gave positive bacterial culture, whereas 5 samples showed no bacterial growth even after 48 hours, which may be due to the consumption of antibiotics by the patients or the presence of another type of causative agents, that might need special technique for their detection such as viruses, chlamydia, and mycoplasma.

A total of 120 bacterial isolates were obtained from the 95 samples collected. From the results it was shown that Grams negative bacteria constitutes 57.5% (69/120) from the total isolates and were considered the predominant aetiological agent of OM compared to gram positive bacteria which constitute 42.5% (51/120 isolates), as shown in figure(3-1).



Figure(3-1) The percentage of Grams negative and Grams positive bacteria among patients with otitis media

3.1.1 *Pathogenicity of bacteria in Otitis Media:*

Sixty eight cases of OM were caused by single bacterial (Table 3-1), where *P.aeruginosa* constitutive (16/68), *M.catarrhalis* (14/68) were

predominant in single causative microorganism followed by *S.aureus* (11/68) , *S.pneumoniae* (9/68).However, each of the following bacteria *K. Pneumoniae*, *S.epidermidis*, *Acintobacter spp* and *Proteus spp* constitutive (4/68) .

Table (3-1)Distribution of bacterial isolates from patients with otitis media according to the isolates

Bacteria	Single isolate	Mixed isolates	Total isolates	%
<i>M.catarrhalis</i>	14	11	25	20.83
<i>P.aeruginosa</i>	16	8	24	20
<i>S.pneumoniae</i>	9	14	23	19.66
<i>S.aureus</i>	11	10	21	17.5
<i>K. pneumoniae</i>	4	2	6	5
<i>Proteus spp</i>	4	1	5	4.16
<i>Acintobacter spp</i>	4	1	5	4.16
<i>S.epidermidis</i>	4	0	4	3.3
<i>S.pyogenes</i>	1	1	2	1.66
<i>E.coli</i>	0	2	2	1.66
<i>H.influenza</i>	0	1	1	0.83
<i>Diphtheroides spp</i>	1	0	1	0.83
<i>Bacteroids sp</i> (Obligate Anaerobic Bacteria)	0	1	1	0.83
Total	68	52	120	100

More over , the aerobic and facultative anaerobic bacteria were found in high frequency (99.17%), compared to anaerobic bacteria which include *Bacteroides* spp (0.83%) .

The main bacterial types isolated in this study were shown in table(3-1) *M.catarrhalis* was the most common bacterial species isolated from ear infections species (20.83%) *M.catarrhalis* infections are encountered more often now than in past in ear infection, such as in Kufa covernorate AL-Dahhan,(2001) reported (1.1)%, in Babylon covernorate AL-Trufei, (2002) reported (3.6)%,and and AL-Timimi,(2004) reported (6.48%).

This propably due to bacterium antibiotics resistance properties, and they are commensals of mucosal surfaces of upper respiratory tract. This bacteria occasionally is causative of opportunistic infection when the patient uses corticosteroid or immune suppressive agents .

Pseudomonas aeruginosa was the second type of bacteria isolated from chronic OM (20%),This result was expected for this organism, due to many reasons, firstly , *P.aeruginosa* is the most secondary invaders when the resistance of the middle ear is lowered .Secondaly, its spread easily to the compromised patients via external canal from healthy carriers or environmental sites .Thirdly, the high incidence of *P .aeruginosa* indicates more general antibiotic resistance than in the case with gram positive strains and fourthly ,its resistance for phagocytosis and opsonization by producing large number of extracellular products such as alkaline protease, elastases and exotoxin A which can cleave IgA lead to inhibit the function of the immune system of the cells , this agree with the result mentioned by Govin (1996), and Stenfors and Raisanen,(1993). Our results were in agreement with Ibekwe *et al.*,(1997) who had found that *P.aeruginosa* present in 22.5% .

Streptococcus pneumoniae which is considered from the microflora of nasopharynx in healthy adults, had constituted (19.7%), as shown in table (3-1), and were considered the third bacteria isolated in this study. These microorganisms were reported by many researchers such as Ibekwe *et al.*, (1997), from the common bacterial species causing acute OM. *S.pneumoniae* was regarded as a pathogen in immunodeficient patient, and produce many virulence factors such as hemolysin, capsule, and IgA protease.

The other bacteria that had been isolated in this study was *S.aureus* which represents (17.5%). This frequency may be due to, firstly, it may enter the middle ear from external canal as a normal flora and by reflux OM when the tympanic membrane was not intact, secondly, *S.aureus* may inherent nature of developing resistant strains for antibiotics. *S.aureus* also contain teichoic acid and lipoteichoic acid, capsular material which facilitates the adherence of these bacteria to epithelium this agree with result mentioned by Yassin, (1990) and, Brook *et al.*, (1998). Brook, *et al.*, (1998) reported that *S.aureus* was the dominant causative agent of CSOM in children older than six years. Our results agree with ALKhafaji (1993), and Aubaid, (1998), and disagreement with Abdullah, (1997) who had found lower number of OM caused by *S.aureus*.

Proteus spp like *P.aeruginosa* were predominantly secondary invaders from external canal and when the resistance of the middle ear is lowered. In this study *Proteus spp* constitute 4.16 % table (3-1). This finding was incompatible with ALUbaydi 2006, and compatible with Ibekwe *et al.*, 1997 who found that *Proteus spp* represented 3.6%.

Klebsiella pneumoniae, and *E.coli* in 5%, and 1.66% respectively, as shown in table (3-1), this may be due to presence of capsular

polysaccharide which plays an important role in pathogenicity of *K.pneumoniae* in OM infection, the finding is in agreement with Yassin (1990) who found that both microorganisms were representing in 6.2 %,and 3.2% respectively, and ALKhafaji (1993),who found that microorganisms were representing in 6.18%,and 2.8% respectively. The finding in agreement with these studies,present study have that *K.pneumoniae* 5% more than *E.coli* 1.66%.

Other gram negative bacteria such as *Acintobacter* was isolated from OM, but mostly in low frequency. *Acintobacter* represent (4.16%), (AL-Trufei,2002),and(AL-Timimi 2004),found that microorganism was representing in 7.62%,and 2.77% respectively.

Strept.pyogenes (1.66%) isolated from OM but in low frequency ,this result agrees with AL-Turfei(2002).

H.influenzae representing 0.83% from total pathogens isolated from acute O.M, Yilman *et al.*,(2006) mentioned that *H.influenzae* regarded a the second pathogen that follow *S.pneumoniae* causes OM. These results were disagree with the recent results may be due to geographical differences, different cultural practices and nutrition and secio – economic factors .

Staph.epidermidis and *Diphthroid bacilli*, as shown in tables (3-1) . These microorganisms were previosly regarded as non pathogenic as they considered as contaminants from the skin. This agrees with El-Shamy (1993) who had emphasized their essential role in chronic OM. These commensals bacteria may have a role as approtunistic pathogens in the presence of weakened local tissue defence when immunosuppressive agents were used, and also the antibiotics had been associated with

emergence of opportunistic infection by microorganisms not previously regarded as pathogenic to man like *S.epidermidis* and *Diphtheroid bacilli*

The mixed bacterial types (52) were related to two different genus and species as shown in table (3-2), this might describe a cooperation between these different species in disease processes. *Sterpt.pneumoniae* (14) isolates were predominant in mixed infection. This in the finding that *Sterpt. pneumoniae* produce protease and also contain capsule. The capsule resistance for phagocytosis and the protease is effective against IgA give support for other bacteria causes OM such as *Staph.aerues*, *P.aeruginosa*, and *M.catarrhalis*.

P.aeruginosa are producing large number of extracellular products such as alkaline protease, elastases and exotoxin A which can cleave IgA lead to inhibit the function of the immune system of the cells, this give support for other bacteria causes OM such as *S.aerues*, *Proteus spp*, and *E.coli*.

K.pneumoniae contain thick capsular polysaccharide which considered resistant to phagocytosis, this support *M.catarrhalis* and *E.coli* for causes OM.

Anaerobic bacteria were mostly isolated in mixed culture with facultative anaerobic bacteria (*S.pyogenes*) and represented in one case as shown in table (3-2). This finding agrees with Ibekwe *et al.*, (1997) who found that anaerobes represent about 0.9% of all isolates. This pattern of mixed culture between aerobic and anaerobic bacteria may be attributed to the synergistic relationship between aerobes and anaerobes, in which the aerobic bacteria removes oxygen, produces substances that lower the potential of the tissues, or provide nutrients that are necessary for the proliferation of oxygen the anaerobic pathogens, whereas anaerobic bacteria alone does not usually establish themselves in a suppurative process.

Table (3-2) Types of bacteria isolated from mixed growth

Mixed growth	NO.
Bacterial isolates	
<i>S.aureus</i> + <i>S.pneumoniae</i>	14
<i>M.catarrhalis</i> + <i>S.pneumoniae</i>	5
<i>P.aeruginosa</i> + <i>M.catarrhalis</i>	2
<i>P.aeruginosa</i> + <i>K.pneumoniae</i>	2
<i>P.aeruginosa</i> + <i>Proteus spp</i>	2
<i>P.aeruginosa</i> + <i>S.pneumoniae</i>	1
<i>P.aeruginosa</i> + <i>E.coli</i>	1
<i>M.catarrhalis</i> + <i>S.aureus</i>	1
<i>M.catarrhalis</i> + <i>Proteus spp</i>	1
<i>M.catarrhalis</i> + <i>K.pneumoniae</i>	1
<i>K.pneumoniae</i> + <i>E.coli</i>	1
<i>M.catarrhalis</i> + <i>E.coli</i>	1
<i>H.influenza</i> + <i>Acintobacter spp</i>	1
<i>Bacteroids sp</i> + <i>S.pyogenes</i>	1

3-2 Virulence Factors

Virulence factors for some bacterial isolated from patients with OM were studied, *M. catarrhalis* were tested for their abilities to produce colonization factors antigen by Haemmagglutination Test (HA). The results showed that most isolates of *M. catarrhalis* (80%) were able to produce colonization factors antigen.

Most strains of *M. catarrhalis* that adherence were mediated through fimbriae or pili enhance attachment to host cells and resistance to phagocytosis to provide a way for the bacterium to evade the hosts immune response .

Capsule was detected in some isolated bacteria by using Hiss methods. The results in table (3-3) reveal that all isolates of *M. catarrhalis* which were isolated from patients with bacterial OM did not possess the polysaccharide capsule. This result is similar to the result observed by Ahmed, *et al.*, (1991) who had pointed out that *M. catarrhalis* could not possess the polysaccharide capsule. All *M. catarrhalis* isolates were found not capable of producing the extracellular hemolysin. Only 4 isolates of *M. catarrhalis* were able to produce siderophores.

Furthermore, it was known that bacteria which were able to produce siderophores have no ability to produce hemolysin, so bacteria need only one mechanism for obtaining iron. Iron can increase disease risk by functioning as a readily available essential nutrient for invading microbial and neoplastic cells, to survive and replicate in hosts, microbial pathogens must acquire host iron, this identical with that result obtained by (Goel and Kapil, 2001).

The ability of *M. catarrhalis* to produce extracellular proteases was investigated and it was found that only 2 (20%) isolates of *M. catarrhalis* were able to produce extracellular proteases. *M. catarrhalis* can produce proteases when reach mucosal surfaces may often encounter secretory IgA, which could inhibit their adherence and growth on epithelium, the bacteria that cause disease on these mucosal surface were able to evade the action of secretory antibody by producing IgA proteases, that inactivate IgA antibody and this enzyme play role in colonization of mucosal surface. The bacteria produce several protease which have the capacity to degrade host proteins releasing amino acid as nutrients and may degrade proteins such as IgA which are involved in host defense, and may also be involved in host tissue damage.

Table (3–3) Type of virulence factors detected in *M. catarrhalis*

No.of Isolates	colonization factors antigen	capsule	Heamolysin	Sidrophores	Extracellular proteases
1	+	-	-	-	+
2	+	-	-	-	-
3	+	-	-	-	+
4	-	-	-	-	-
5	+	-	-	+	-
6	+	-	-	+	-
7	+	-	-	-	-
8	+	-	-	-	-
9	+	-	-	+	-
10	-	-	-	+	-
Total	80%	0	0	40%	20%

The ability of *P. aeruginosa* isolates to produce some virulence factors was investigated and the results are shown in table (3-4), the isolates were tested for their abilities to produce adherence factors, and it was found that 5 bacterial isolates were able to produce adherence factors, this may be due to the presence of fimbriae, the fimbriae of *P. aeruginosa* will adhere to epithelial cells of the upper respiratory tract and causes OM, this identical to result obtained by Mouricout,(1997).

Also, it was found that 60% of *P. aeruginosa* isolates were possessing the polysaccharide capsule which surrounded the bacterial cell, capsular polysaccharide can protect *P. aeruginosa* against the ciliary action of the respiratory tract and complement mediated lysis, this agree to the result mentioned by Goodier and Lodei, 2000.

The ability of *P. aeruginosa* to produce hemolysin was investigated and it was found that only 3 isolates of *P. aeruginosa* were able to produce extracellular hemolysin. *P. aeruginosa* produce two hemolysin, it appears

to be cytotoxic for most eukaryotic cells, hemolysins contribute to invasion through their cytotoxic effects on eukaryotic cells. *P. aeruginosa* isolates were also tested for their abilities to siderophores synthesis. The results showed that all isolates of *P. aeruginosa* were able to produce siderophores, and also, it was shown that only 3 isolates of *P. aeruginosa* can produce extracellular proteases. *P. aeruginosa* could produce of large number of extracellular proteases such as alkaline protease, elastases, and exotoxin A which can cleave IgA which then lead to inhibit the function of the cells of the immune system, thus *P. aeruginosa* is resistant for phagocytosis and opsonization, this agrees with that result mentioned by Stenfors and Raisanen, (1993). Weber and Osborn (1982) also found that toxin A and elastase inhibit protein synthesis in macrophage of mice thus directly effect phagocytosis.

Table (3–4) Type of virulence factors detected in *P. aeruginosa*

No. of Isolates	colonization factors antigen	capsule	Hemolysin	Siderophores	Extracellular proteases
1	-	-	+	+	-
2	+	-	-	+	-
3	+	+	-	+	+
4	-	+	-	+	-
5	+	+	+	+	-
6	-	-	-	+	+
7	-	+	-	+	+
8	+	+	+	+	-
9	+	+	-	+	-
10	-	-	-	+	-
Total	50%	60%	30%	100%	30%

The ability of *S. pneumoniae* isolates to produce some virulence factors were investigated. *S. pneumoniae* isolates were tested for their abilities to

produce colonization factors antigen as shown in table(3-5),and it was found that all isolates were able to produce colonization factors antigen in the presence human blood (group A) .

Further more ,it was found that only 4 isolates from 6 isolates of *S.pneumoniae* 66.6% had capsular polysaccharide ,The capsular polysaccharide is very essential virulence factors through protecting the organisms from complement activation and phagocytosis mediated destruction.

Additionally, the detection of hemolysin in blood agar was also studied. It was found that all *S. pneumoniae* isolates produced hemolysin, and the type of hemolysis was Alpha-hemolysis due to the presence of a green zone of hemolysis that surrounded the bacterial colonies formed on blood agar. *S. pneumoniae* produced an Alpha-hemolysin that reduced hemoglobin (red) to methemoglobin (green), caused a greenish zone to surround the colony ,The same results were obtained by Facklam and Pigott, (1994).The function of hemolysin is to provide the microorganism with iron and it makes the bacteria unable to produce any factor for obtaining the iron from environment.Moreover,the ability of *Strep. pneumoniae* to siderophores synthesis was studied, and it was found that all isolates of *S. pneumoniae* could not produce siderophores or iron-chelating factors under low-iron conditions and could use either hemin or hemoglobin as a sole source for the required iron. This result similar with the result obtained by Tai, *et al.*, (1993) . The ability of *S. pneumoniae* to produce extracellular proteases was investigated and it was found that only one isolates of *S. pneumoniae* were able to produce extracellular proteases. *S. pneumoniae* can produce immunoglobulin A1(IgA1) proteases, and these proteases are of the serine type which enables *S. pneumoniae* to

evade the protective functions of the principal immunoglobulin isotype of the upper respiratory tract, these proteases degrade human immunoglobulin A1, as well as on the balance between secreted and cell-associated forms of the enzyme. These results were similar to those obtained by Poulsen *et al.*, (1996) and Reinholdt and Kilian, (1997).

Table (3–5) Type of virulence factors detected in *S. pneumoniae*

No.of Isolates	colonization factors antigen	capsule	Heamolysin	Sidrophores	Extracellular proteases
1	+	-	+	-	-
2	+	-	+	-	+
3	+	+	+	-	-
4	+	+	+	-	-
5	+	+	+	-	-
6	+	+	+	-	-
Total	100%	66.6%	100%	0	16.6%

The ability of *Staph.aureus* isolates to produce some virulence factors was investigated and the results of virulence factors are shown in table (3-6). *S. aureus* isolates were tested for their abilities to produce colonization factors antigen, and it was noticed that 4 bacterial isolates could produce colonization factors antigen. Several studies had shown that *S.aureus* strains could adhere to host tissues through teichoic acid, this acid was one of the most important component in bacterial cell wall. However, the ability to produce additional adherence factors would help the bacteria to colonize various tissues.

Moreover, some strains of *S. aureus* 10% had shown to possess the polysaccharide capsule which was an important component in the pathogenesis, and enhances bacterial virulence by modulate *S. aureus*

adherence to endothelial surface in vitro, animal studies suggest that it also promotes bacterial colonization and persistence on mucosal surfaces, this agree with result mentioned by Nair *et al*,2000.

All *S. aureus* isolates had the ability to produce the hemolysin, and the type of hemolysis was Beta-hemolysis due to the presence of a clear zone of hemolysis around the bacterial colonies formed on human blood agar, this agree with result mentioned by Dinges,*et al.*, 2000.

S. aureus was also tested for it's ability to siderophores synthesis, and the results showed that all isolates of *S. aureus* were not able to produce siderophores. The production of hemolysin would make the bacteria does not need the production of siderophore for obtaining iron because the former would help it to gain the iron.

The ability of *S. aureus* to produce extracellular proteases was investigated, and it was found that 2 isolates of *S. aureus* were able to produce extracellular proteases, but other bacterial isolates, 8 isolates could not produce this enzyme. The production of extracellular protease varied considerably among clinical isolates of *S. aureus*, and the presence of the major protease genes, in all the protease-negative strains analyzed suggested that the lack of protease production was due to some regulating host cell factors. This identical with result mentioned by Karlsson and Arvidson, (2002) .

Table (3–6) Type of virulence factors detected in *S. aureus*

No.of Isolates	colonization factors antigen	capsule	Heamolysin	Sidrophores	Extracellular proteases
1	+	-	+	-	-
2	+	-	+	-	+
3	-	-	+	-	+
4	-	-	+	-	-
5	+	+	+	-	-
6	-	-	+	-	-
7	-	-	+	-	-
8	-	-	+	-	-
9	+	-	+	-	-
10	-	-	+	-	-
Total	40%	10%	100%	0	20%

Regarding to *K. pneumoniae* isolates to produce some virulence factors was investigated and the results are shown in table (3-7). The isolates were tested for their abilities to produce colonization factors antigen, and it was found that 3 of *K.pneumoniae* isolates were able to produce colonization factors antigen due to the positive reaction with human blood cells (groupA). *K. pneumoniae* strains had the ability to possess type 3 pili, which was an important factor in adherence of this type of bacteria to mucous of respiratory tracts. These results was identical with the results obtained by Venegas, *et al.*, (1995) and Martino, *et al.*, (1996) and Hornick, *et al.*, (1999).

From the results in table (3-7), it could be noticed that all *K. pneumoniae* isolates were also possessing the polysaccharide capsule which surround the bacterial cell. This result agrees with the result obtained by Podschun and Ulmann, (1998) who had pointed that the diagnostic feature for *K. pneumoniae* is capsular polysaccharide , and it's essential virulence factor for *K. pneumoniae*.

The results also showed that all *K. pneumoniae* isolates could not produce hemolysin extracellularly when cultured on blood agar, and the type of hemolysis was gamma hemolysis because there was no hemolysis present on blood agar.

Klebsiella pneumoniae isolates were also tested for their abilities to synthesize siderophores. The results showed that all isolates of *K. pneumoniae* were able to produce siderophores, all *Klebsiella* strains have the ability to possess a siderophore system, and *K. pneumoniae* depend on this system for the uptake of iron which may be considered an essential factor in bacterial growth. Thus, production of siderophores may give access to both sources of iron, resulting in enhanced growth in the host. From the results obtained in this study it was shown that the bacteria can only produce either hemolysin or siderophores, not both.

Also, the ability of *K. pneumoniae* isolates to produce extracellular proteases was investigated and it was found that 3 isolates of *K. pneumoniae* could produce extracellular proteases. Proteolytic enzymes were very important factors in which the bacteria can degrade protein and then cause penetration to the tissues, this is identical with that result obtained by Raksha *et al.*, (2003).

Table (3–7) Type of virulence factors detected in *K. pneumoniae*

No. of Isolates	colonization factors antigen	capsule	Hemolysin	Siderophores	Extracellular proteases
1	+	+	-	+	+
2	+	+	-	+	+
3	-	+	-	+	+
4	+	+	-	+	-
Total	75%	100%	0	100%	75%

3.3.Detection of Uronic acid

Uronic acid in 22 samples of ear discharges were selected on the basis of the presence of mucoid discharge and investigated by using colorimetric method (table 3-8). It was found that uronic acid was detected in most samples of ear effusion taken from *K. pneumoniae*, *S. pneumoniae* and *P.aeruginosa* isolates. This may be attributed to the possession of these bacteria to capsular polysaccharides, which may be secreted extracellularly as a uronic acid.

The size of capsules also may have effect on the mount of the uronic acid produced the bacteria. So, the bacteria with small capsule have a little amount of production of this acid .

Uronic acid was not detected with the isolates of *M. catarrhalis* when taken the ear sample (infection without discharge) as a controlling group. This may be attributed to *M. catarrhalis* had not capsular polysaccharides to produce effusion (we conclude that bacterial isolated which produce ≤ 10 Microgram/ml concentration of uronic acid considered less amount of uronic acid and not capable to produce capsule).

Uronic acid can protect the bacteria from secretory IgA produced by mucosal surface of upper respiratory tract infection, therefore uronic acid important in pathogenicity of bacteria that causes OM infection and ear discharge. *P.aeruginosa* were mainly isolated from chronic OM infection , and were characterized by mucoid effusion . This effusion were may be due to alginate which is the main character for the production of uronic acid, this agree with result mentioned by Stevens *et al.*, (1984) and Alberch and Schiller, (2005) and Deretic, *et al.*, (1989) .*P.aeruginosa* isolated from patients with OM had a mucoid colony morphology due to overproduction of the alginate, which contribute to the persistence of

bacteria in patients this agree with mentioned by Jain and Ohman (1998). The control of the production of uronic acid by bacteria in ear effusion by a regulator genes, these indicated that genes responsible from uronic acid production found on bacterial chromosome or plasmid, those genes role play important in increased the acid production .

Table(3–8)Concentration of uronic acid in isolated bacteria

NO.	Isolates	Present of capsules	Concentration of uronic acid Microgram/ml
1	<i>Streptococcus pneumoniae</i>	+	22
2		+	29
3		+	15
4		+	28.9
5		+	27.6
6		+	41.5
7		+	31.4
8		+	75
1	<i>Pseudomonas aeruginosa</i>	+	15
2		-	4.4
3		+	15
4		+	36.6
5		+	56.2
6		+	33
7		-	5.4
1	<i>Klebsiella pneumoniae</i>	+	61.2
2		+	18.4
3		+	16.4
1	<i>Staphylococcus aureus</i>	+	48
2		-	8.5
3		+	17.5
1	<i>Moraxella catarrhalis</i>	-	4.4

Control ≤ 10

3.4 Effect of Some Antibiotics on Bacterial isolates

The effects of different antibiotics on bacterial isolates was investigated.

The effect of Amoxicillin on bacterial strains was studied as shown in figure (3-2). The results showed that all isolates of *P.aeruginosa* and *K. pneumoniae* were completely resistant (100%) to Amoxicillin, whereas *S. aureus* and *M. catarrhalis* were resistance (90% and 80%) respectively, and also (50%) of *S. pneumoniae* isolates were resistant to amoxicillin. These results of high resistant to this antibiotic in nearly compatible with that of AlGibouri(2006) who found that 90% of *P.aeruginosa* were resistant to Amoxicillin , AlSaedi (2000) was found that 98.2% of *K. pneumoniae* were resistant to Amoxicillin ,

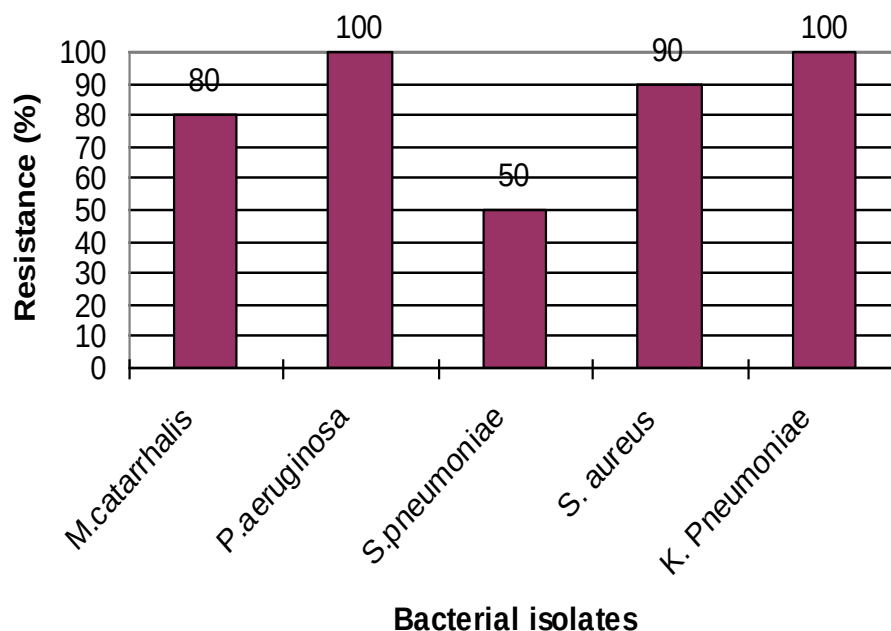
Varon,*et al.*, (2000) found that *M. catarrhalis* were fully susceptibility to amoxillin.

S. aureus were resistant exclusively to amoxillin were attributed to their production of beta-lactamase enzymes that break down the beta lactam ring and render it inactive, this is mediated by extra-chromosomal piece of DNA (plasmid). Or by decreasing membrane permeability to words the antimicrobial agents, This result agree with result mentioned by Barid, (1996)

This result was also higher than that the result obtained by Soh, *et al.*, (2000) who clarified that the resistance rate of *S. pneumoniae* to Amoxicillin was (70%).

the primary mechanism for resistance to β -lactam is the enzymatic hydrolysis of the β -lactam ring, failure of antibiotic to penetrate to

pencillin binding proteins (PBP) target site and low affinity binding of antibiotic to PBP also confer resistance to these antibiotic, This result was similar with those asserted by Ang *et al.*, (2004)



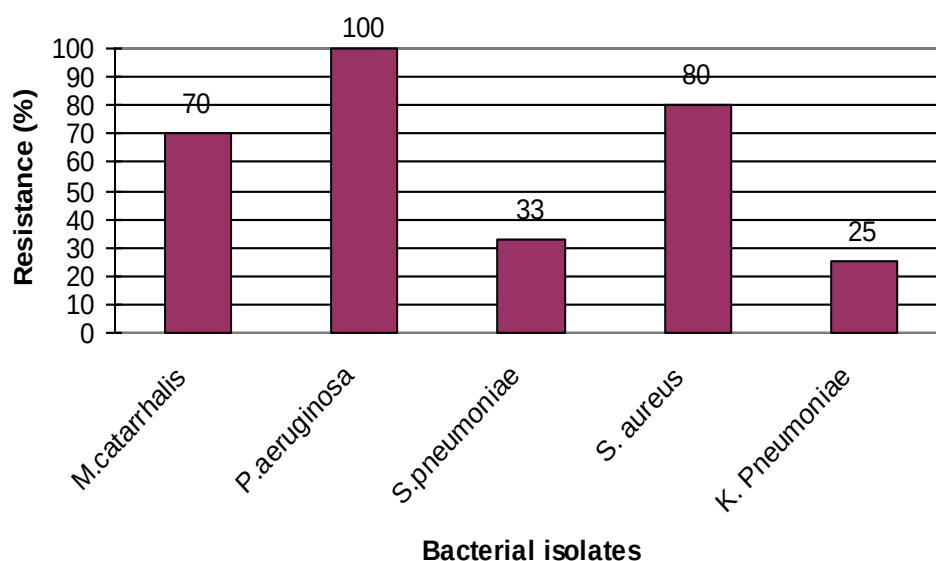
Figure(3-2) Effect of Amoxicillin on bacterial isolates

Regarding to ,Cefotaxime which is third generation of cephalosporin, as shown in figure (3-3).

The results showed that all isolates of *P.aeruginosa* were resistant (100%) to cefotaxime. *M. catarrhalis* and *S. aureus* isolates were resistance (70% and 80%) respectively. The resistance decreased gradually to (33% and 25%) of *S. pneumoniae* and *K. pneumoniae* respectively.

These results was in agreements with that Gums and Gainesville, (2000) who found that (29.3%) of the *S. pneumoniae* were resistant to cefotaxime,

On the other hand, Hanumanthappa *et al.*, (2003) had pointed out that (82.4%) of *S. aureus* strains were resistant to cefotaxime .



Figure(3-3) Effect of Cefotaxim on bacterial isolates

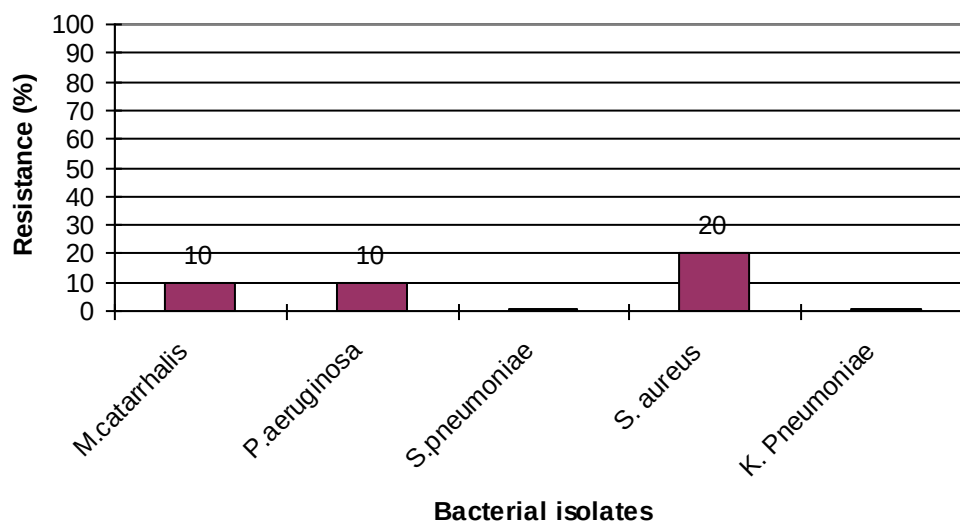
Al-Timimi(2004) found that 71.5% of *M.catarrhalis* were resistance to Cefotaxime .The resistance of *P. aeruginosa* isolates to cephalosporin may be due to the synthesis of β -lactamase as well as loss of PBP by mutation confer resistance to this antibiotics. *P. aeruginosa* exhibits resistance to many antibiotics possibly due to the intrinsic or acquired in which these bacteria are highly inherently resistance and this arises from combination of unusually restricted outer membrane permeability and chromosomally encoded β -lactamase. This agrees with result mentioned by Hankok and Speert (2000) and Bisiklis *et al.*,(2005)

The resistance of *K. pneumoniae* to cefotaxime is probably attributed to the fact that most clinical isolates of *K. pneumoniae* produce sixty different plasmid and/or chromosomal mediated beta-lactamase enzymes. As plasmid mediated enzyme production is much more easily transferred to cells, there had been increase in beta-lactamase resistance to third generation cephalosporins (i.e. cefotaxime). These enzymes are called “Extended Spectrum Beta-lactamses” and these enzymes are

currently inhibited by clavulanic acid. This agree with result obtioned by Masters, (1997) Moreover, new generations of β -lactam antibiotics are better to be used instead of ampicillin and amoxicillin like those containing β -lactamase inhibitors, namely clavulanic acid .

Regarding ciprofloxacin (figure 3-4) , most bacterial isolates were susceptible to ciprofloxacin *S. pneumoniae*, and *K. pneumoniae* were completely susceptible(100%)to ciprofloxacin, followed by *M.catarrhalis*, *P.aeruginosa* and *S. aureus* were susceptible to this antibiotic.

These results were agreement with those obtained by Martinez, *et al.*, (2004) who found that all isolates of *S.aureus* which isolated from patients with bacterial OM were susceptible to Ciprofloxacin. Ciprofloxacin was only drug of choice against *S. aureus* due to blocking of DNA gyrase , this agree with result obtained by Rashmi *et al.*,(2005).



Figure(3-4)Effect of Ciprofloxacin on bacterial isolates

P. aeruginosa possesses active efflux pump system acts as wide transporters for a whole rang of antibiotics , that coupled with the narrow

porin channels in the outer membrane of this organism, restrict diffusion of many antimicrobial agent into the cell, These results were identical with those obtained by (Schweizer, 1998).

The results were similar to the results obtained by Martinez, *et al.*, (2004) who had found that (6.5%) of *S.pneumoniae* strains isolated from patients with bacterial OM are resistance to ciprofloxacin.

Nichol, *et al.*, (2003) showed that the susceptibility of *S.pneumoniae* isolates to ciprofloxacin, in fact, had demonstrated less genetic variability than isolates with reduced susceptibility to ciprofloxacin. So these results suggest that the emergence of ciprofloxacin-resistant *S. pneumoniae* was currently not attributable to clonal dissemination.

The results was in agreement with Tonic, *et al.*, (2005) found that (4%) of *K.pneumoniae* were resistance to ciprofloxacin.

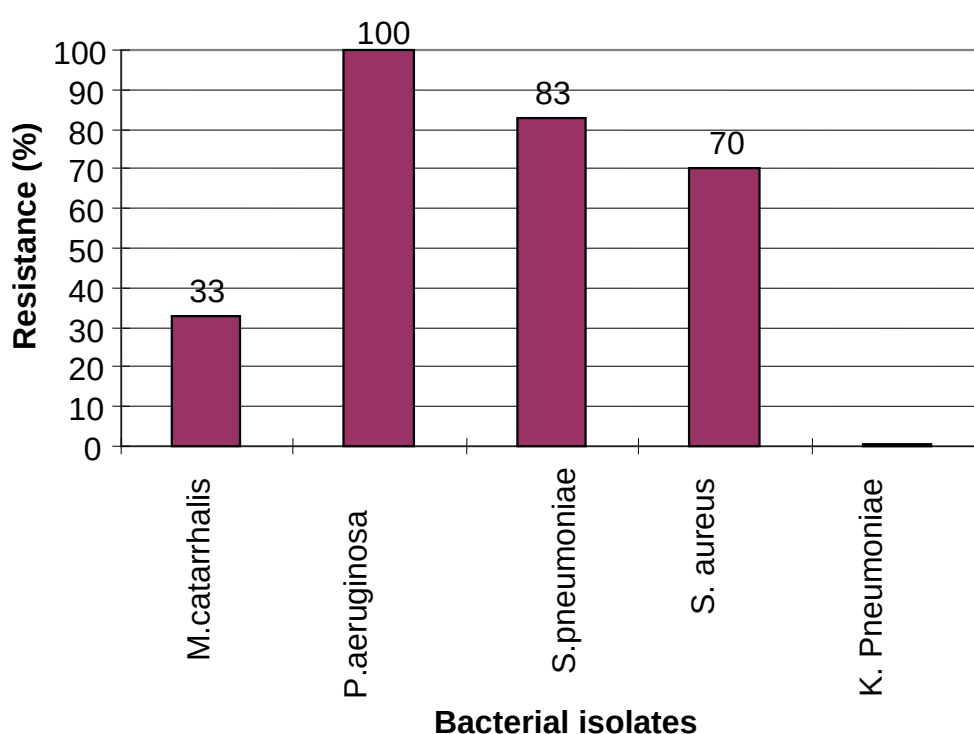
All *K. pneumoniae* strains isolated from patients with bacterial otitis media were susceptible to Ciprofloxacin, and have suggested that the fourth generation of fluoroquinolones seem to be more effective than previous generations of fluoroquinolones against *K. pneumoniae* strains and other organisms isolated from patients with bacterial otitis media , Our results agrees with result pointed out by Lima, *et al.*, (2004) .

ciprofloxacin was bactericidal drug,they affected against gram negative and gram positive bacteria and the resistance to fluoroquinolones was through chromosomal mutation or alternation affecting the ability to fluoroquinolones to permeate to bacterial cell wall.

Rrgarding Trimetheprime-sulfamethoxazole (figure3-5),most bacterial isolates showed resistance to Trimetheprime- sulfamethoxazole; *P.aeruginosa* were completely resistant and only *K. pneumoniae* was fully susceptible to this antibiotic. About 30% of *M. catarrhalis*, 70% of

S. aureus and 83% of *S. pneumoniae* were resistant to this antibiotic. This result agrees with AlGiburi(2006) who found that 90% of *P.aeruginosa* were resistant to this antibiotic, and Karlowsky *et al.*,(2003) who pointed out that most *P.aeruginosa* were resistance to this antibiotic.

A combination of Trimetheprime and sulfame thoxazole inhibits bacterial growth by inhibiting synthesis of dihydrofolic acid , its antibacterial activity includes some Gram negative and gram positive aerobic excepting *P.aeruginosa*.



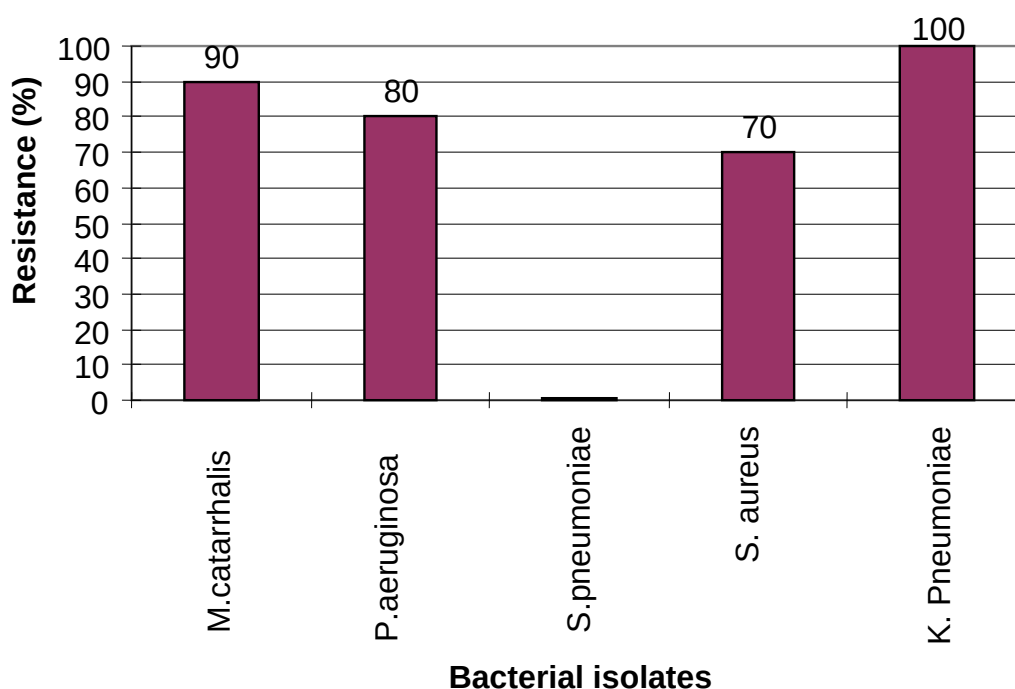
Figure(3-5)Effect of Trimethoprim- sulphamethoxazol on bacterial isolates

Doxycyclin (figure 3-6), most bacterial isolates showed resistance to doxycyclin ; only *S. pneumoniae* were completely susceptible to this antibiotic. All isolates of *K. pneumoniae* was resistance to doxycyclin. *S.*

aureus, *P.aeruginosa* , and *M. catarrhalis*, were resistance (70% , 80% , and 90%) respectively .

This result was in agreement with AlGibouri (2006) who found that all isolates of *P.aeruginosa* were resistance to doxycyclin.

Brook *et al.*,(2004) had mentioned that doxycyclin is bacteriostic antibiotic,it is active against wide rang of bacteria, doxycyclin attach to tRNA and by that the prevent complexation of aminoacyl tRNA with mRNA of 30 S unit.



Figure(3-6) Effect of Doxycyllin on bacterial isolates

Furthermore, Erythromycin, (figure 3-7), all *P.aeruginosa*, and *K.pneumoniae*,were resistance to erythromycin, lesser resistance rate to erythromycin were 40% of *S. pneumoniae* and *S. aureus*, whereas 80% of *M. catarrhalis* were resistance to this antibiotic

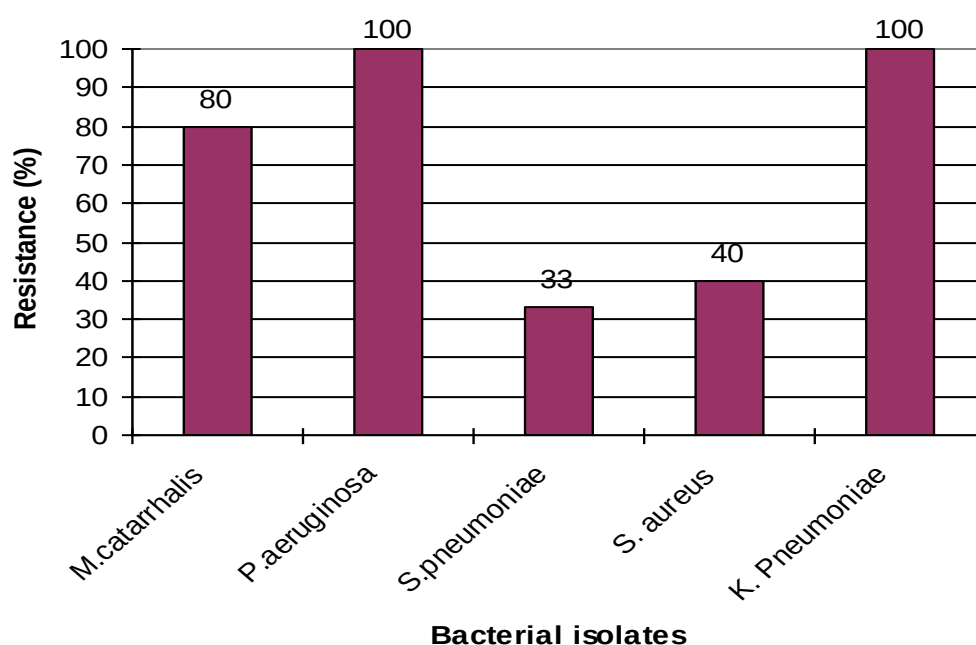
This result was nearly compatible in agreement with the result obtained by Chin, *et al.*, (1999) who had found that (5%) of *S. pneumoniae* were resistance to erythromycin.

Also, Chivez, *et al.*, (2002) had found that (4.2%) of *S. pneumoniae* were resistance to erythromycin.

Chigbu and Ezeronye, (2003) mentioned that (32.6%) of *S. aureus* were resistance to Erythromycin.

Also, the resistant to Erythromycin results from an alteration (methylation) of the rRNA receptor, this is under control of a transmissible plasmid (Bahal and Nahata, 1992).

The result was similar to the result observed by AlGibouri (2006) who found that all of the *P.aeruginosa* were resistant to Erythromycin.



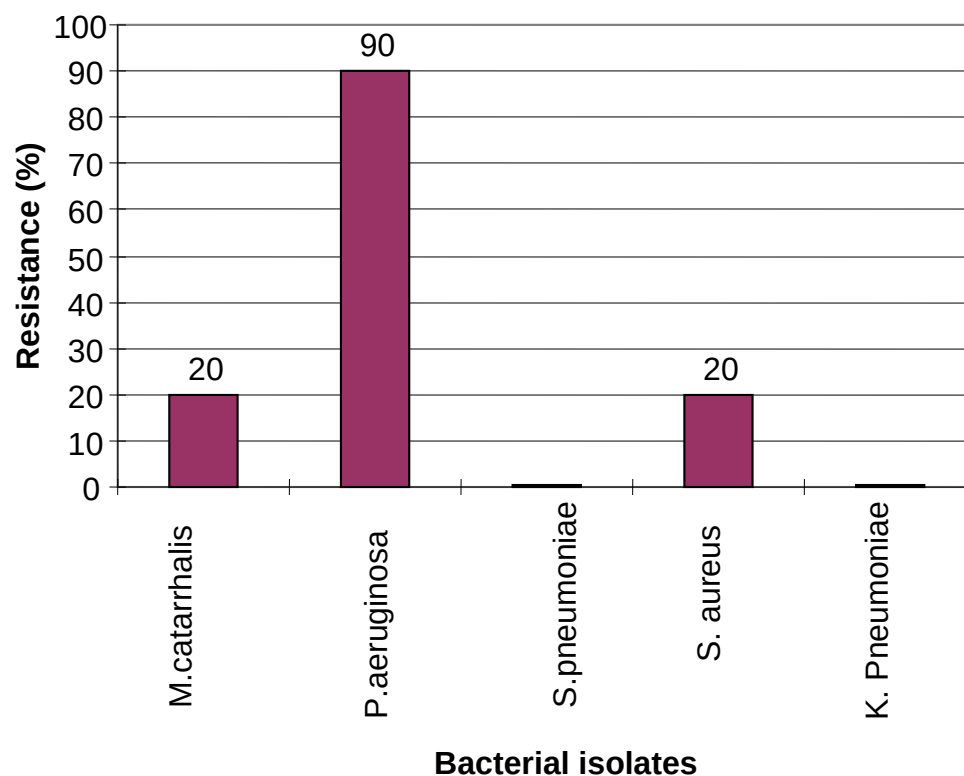
Figure(3-7) Effect of Erythromycin on bacterial isolates

Regarding Clarithromycin,(figure3-8), most bacterial isolates showed susceptible to, all isolates of *Strept. pneumoniae* and *K.pneumonia* were susceptible to clarithromycin. About 20% of *M. catarrhalis* and *Staph. aureus* were resistance to clarithromycin , while 90% of *P.aeruginosa* were resistance to this antibiotic.

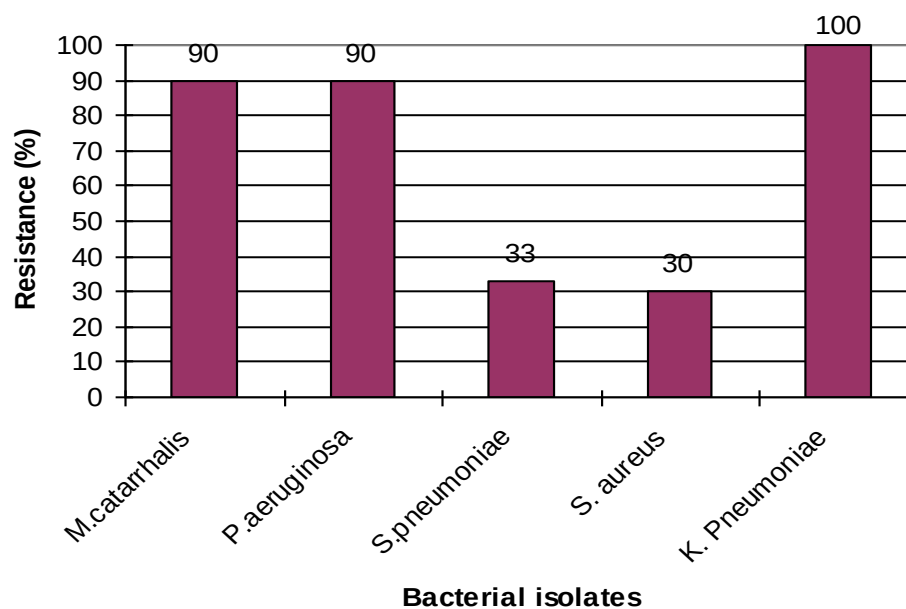
Azithromycin (figure 3-9), all isolates of *K. pneumoniae* were completely resistance to this antibiotic. About 90% of *M. catarrhalis* and *P.aeruginosa* were resistant to this antibiotic. Other isolates showed resistance in lesser degrees 20 % of *S. pneumoniae* and *S. aureus* were resistant to Azithromycin. Azithromycin, and Clarithromycin from the macrolides (azalides) group of antibiotics is chemically related to erythromycin and it was active against staphylococci and streptococci, interferes with bacterial ribosomal function and inhibits protein formation. It is active against Gram-negative bacteria but is relatively less effective against Gram-positive type than Erythromycin .

The newer macrolides (Azithromycin and clarithromycin) were very beneficial in the management of OM infection.

Imamura, *et al.*,(2005) showed that azithromycin has bactericidal activity against *P. aeruginosa*, Azithromycin directly disrupted the outer membrane of *P. aeruginosa*, probably by acting to competitively displace of divalent cations from their binding sites on LPS on the outer membrane, azithromycin was capable of permeabilizing the outer membrane of *P. aeruginosa* and that this action was antagonized by Mg^{2+} ,This direct action of azithromycin may contribute to its bactericidal activity against *P. aeruginosa*.



Figure(3-8) Effect of Clarithromycin on bacterial isolates



Figure(3-9) Effect of Azithromycin on bacterial isolates

3.5. Sex and Age Related disease:

After complete identification of causative microorganisms involved in inflammatory OM were analysed according to different factors that may have possible effect for the activation of OM, distribution of patients according to sex and age were observed. The prevalence of OM was higher among females patients than males (54 and 46 respectively) as shown in table (3-9).

The results had indicated that the susceptibility of females to infection with OM was nearly similar to that males, in other words, there is no significant difference ($p > 0.05$) between females (54%) and males (46%) individual with OM, our finding is in agreement with the results of Mak *et al.*, (2000) and AL-Dahhan (2001), since they reported that there was no significant difference in the incidence of OM in relation to sexes, while AlMola *et al.*, (1998), and ALDulumi (2002) results were in disagreement with our results since they indicated that males were affected with OM more than females.

The distribution of patients with OM according to the ages were also studied and were found that the prevalence of this disease was high in the age groups ranging from 1-10 years old; followed by the age from 11-20 years old, and the rest age groups had less frequency than mentioned above, table (3-9). These results were in agreement with the results obtained by Ikeh *et al.*, (1993), and AL-Uboydi (2006) who had noticed that the prevalence of OM increased during childhood. The highest prevalence of OM were among the age group (1-10) years may be attributed to the high incidence of upper respiratory tract infections in this young age group with that possibly resulted and to the shorter, wider, and horizontal Eustachian Tube in children than in adult, offering a greater

opportunities for pathogens to ascend from nasopharynx to sterile middle ear, same result obtained by Bauchner *et al.*,2006

In another view , the higher incidence of OM in children may be secondary to upper respiratory tract infection with bacteria such as sinusitis, which may follow a viral that may destroy the mucociliary barrier and thus permit a secondary invasion by pathogenic bacteria , this result mentioned by Berman, 1995 .

Table (3-9)Distribution of patients with otitis media according to the Sex and age group

Age group	Sex		NO.	%
	Male	Female		
<1-10	22	22	44	44%
11-20	9	12	21	21%
21-30	2	6	8	8%
31-40	2	4	6	6%
41-50	2	6	8	8%
51-60	7	2	9	9%
61-70	2	2	4	4%
Total	46	54	100	100%

References :

- Abdullah,A.H.(1997). Pre-and-post operative bacteriology of chronic suppurative otitis media, prospective study done in ENT-Department in Al-Rasheed Military Hospetal.Fellowship thesis scientific council of Oto. Iraqi commission for medical Specialization in otolaryngology
- Ad Dahhan, H.A. (2001). Bacteriological and Enzymatic study on patients with chronic suppurative otitis media, thesis of M.Sc. College of Science .Unversity of Kufa.
- Ad Dahhan, H.A.;Naher, H.S. and Shubber,E.K.(2005). Isolation and Identification of Bacterial chronic suppurative otitis media, Kufa covernorate .J. college of medicine Al-Qadisial University Diwaniah,IraqV1(2),62-67.
- Adderson, E.E.(1998) Preventing otitis media: medical approaches. *Pediatr.Ann.*27(2):101-107.
- Ahmed, K.; Rikitomi, N.;Ichinose, A.;and Mastsumoto, K. (1991). Possible Precence of capsule in *Branhamella catarrhalis*. *Microbial.Immunol.*35:361-366.
- Alaeus, A. and Stiernstedt, G.(1991) B.C. septicemia in an immunocompetent adult . *scand J infect .Dis* 23:115-116 .
- Albrecht, M.T. and Schiller,N.L.(2005). Alginate Lyase(AlgL) activity is Required for Alginate biosynthesis in *Pseudomonas aeruginosa* .*J.Bacteriology* :187(11)3869- 3872.
- AlDilumi, A.A.;Al Gafri,A.H.;Naserullah,B.A.and AlDilumi,H.H.(2002).

- Bacterial otitis media in Baghobh covernorate. Journal AlNafh(10).
- Aliphas, A.; Prufer, N. and Grundfast, K. M. (2006) Emerging therapies for the treatment and prevention of otitis media. Expert Opin Emerg Drugs 11(2): 251-264
- AlGibouri, L. F. H. (2006). The Resistance of *Pseudomonas aeruginosa* isolated from clinical cases to antimicrobials thesis of M.Sc. College of medicine. University of Babylon.
- AlKhafaji, F. H. (1993). Clinical and microbiological study in chronic suppurative otitis media . Master thesis , College of medicine . University of Baghdad .
- Al-Mahmood , N . M . and Al-Karagohli , A. A . (1999) . Isolation and identification of bactrio in CSOM using prerduced middle ear swab . Iraqi J . comm . Med ., 12:130-132.
- AL-Mola , G. A.; Jassim, T. M. and Al Adhami S. A. (1998). Bacteriological study of otitis media amog children in saddam General Hospital (SGH) in Tikrit city. Med .J. of Tikrit University , 4: 108-111.
- Alonsodevelasco, E.; Verheul, A. F.; Verhoef, J. and Snippe, H. (1995). *Streptococcus pneumoniae*: Virulence factors, pathogenesis and Vaccines. *Microbiol. Rev.* 59 (4):591 – 603
- Alper , C . M .; Dohar , J. E . and Galhan , M . (2000) . Treatment of chronic suppurative otitis media with topical tobramycin and dexamethasone . Arch , Otolaryngol . Head – Neach Surg., 126: 165-173 .

- Al-Saedi, I. A. B.(2000). Isolation and Identification of *Klebsiella pneumoniae* from various infections and detection of some virulence factors associated in their pathogenicity in Hilla province.MSc.Thesis.College of Science. Babylon University.
- AlTimimi, B.J.(2004) Bacteriological study on *Moraxella catarrhalis* isolation from chronic suppurative otitis media patients in Babylon covernorate, thesis of M.Sc. College of Science. Unversity of AlMustansiriya.
- Al Turfei, B.A. (2002). Isolation and Identification of some Bacteria &Fungi causing Ear Infection, thesis of M.Sc. College Science .Unversity of Babylon
- AlUbaydi, B.M. (2006). Bacterial otitis media in Diyala , thesis of M.Sc. College of Education .Unversity of Diyala
- Al-Zaag, A. (1994). Molecular biology of bacterial virulence. 1sted. Baghdad University..
- Amako, K.; Meno, Y. and Takade, A. (1988). Fine structures of the capsules of *Klebsiella penumoniae* and *Escherichia coli* K1. *J. Bacteriol.* 170: 4960 - 4962.
- Ang,J.Y.;Ezike, E. and Asmar,B. (2004). Antibacterial resistant. Indian. J. pediatrics. 71 (Issue-3):229-239.
- Arbeit, R. D.; Karakawa, W. W.; Vann, W. F. and Robbins, J. B. (1984). Predominance of two newly described capsular polysaccheride types among clinical isolates of *Staphylococcus aureus*. *Diag. Microbiol. Infect. Dis.* 2: 85 - 91.

- Archer, G. L. (1998). *Staphylococcus aureus*: a well-armed pathogen. *Clin. Infect. Dis.* 26: 1179 - 1181.
- Arguedas, A. ;Dagan, R. and Leibovitz, E. (2006).A multicenter, open label, double tympanocentesis study of high dose cefdinir in children with acute otitis media at high risk of persistent or recurrent infection. *Pediatr Infect Dis .J.* 25(3): 211-218.
- Arola, M.;Ziegler, T.and Ruuskanen, O. (1988). Rhino virus in acute otitis media .*J pediatrics.*113: 693-695 .
- Arroll, B. (2005). Antibiotics for upper respiratory tract infections: an overview of Cochrane reviews. *Respir Med* 99 (3): 255-261.
- Aubaid,Q.N.(1998). Aerobic bacteria associated with chronic ear discharge *Journal of Babylon University, Iraq* V3 (3).281-284.
- Austriain, R.;Gorbach ,S.L.; Bartlett ,J.G, and Blacklow. (1998). *Infectious Disease. Second edition.*W.B.sauners company .
- Bahal, N. and Nahata, M. C. (1992). The new macrolide antibiotics: Azithromycin,clarithromycin,dirithromycin and roxthromycin. *Ann. Pharmacother.* 26: 46.
- Barbara, P. and Homeier, M.D.(2005) . about middle ear enfections .*J. paediatr childhood*
- Barid, D. (1996). *Staphylococcus*: cluster-forming gram-positive cocci. Mackie and MaCartney practical medical microbiolog. Edited by: Collee, J.G.; Fraser, A. G.;

- Marmino, B. P. And Simons, A. 14th ed., The Churchill Livingstone, Inc. USA. 245 – 261.
- Baron, E. J.; Peterson, L. R. and Finegold, S. M. (1994). Bailey and Scott's Diagnostic Microbiology. 9th ed., The C.V. Mosby Company, U.S.A .
- Bauchner H, Marchant CD, Bisbee A, (2006) Effectiveness of Centers for Disease Control and Prevention recommendations for outcomes of acute otitis media. *Pediatrics* 117(4):109-1017
- Berman, S. (1995). Management of acute and chronic otitis media in Pediatric Practice. *Curr. Opin. Pediatric.*, 7:513-522.
- Proteolytic Enzymes, R.H. and Bond, J.S. (1989). *enzymes: A practical approach*. Academic press. Oxford.
- Bisiklis, A.; Tsiakiri, E. and Alexiou-Danieel, S. (2005). Phenotypic detection of *Pseudomonas aeruginosa* producing metallo- β -lactamases. *European Society of clinical microbiology and infectious disease, 15th European congress of clinical microbiology and infectious disease*, 1134:1-11.
- Bliss, J.M. and Silver, R.P. (1996). Coating the surface: a model for expression of capsular polysialic acid in *E. coli* K1. *Microbiol.* 21:221-231.
- Bluestone, C.D.; Gorbach, S.L.; Bartlett, J.G.; and Blacklow, N.R. (1998). *infectious diseases .second edition*. W.B. Saunders Part 1: p 530-550 .

- Boyl, M.D.P.(1995). Variation of multifunctional surface binding proteins-A virulence strategy for group A *Streptococci*.J.Theor.Biol.173(23):415-426
- Brook,I.;Yocum, P. and Shah,K.(1998). Aerobic and Anaerobic Bacteriological of otorrhea associated with tympanostomy tubes in children. Acta. Otolaryngol., 118: 206-210.
- Brook,I.(1998). Microbial factors leading to recurrent upper respiratory tract infections .Pediatr.infect.Dis. J.17:562-567
- Brooks, G.F.; Butel, J.S. and Morse, S.A. (2004). Jawetz, Melnick and Aldelberg's Medical Microbiology. 23rded. Lange Medical Books, McGraw- Hill
- Brownlee, R.E.; Hulka, G.F.:Prazma, J. and Pillsaury, H.C.(1992). Ciproflaxacin: use as a topical otic preparation. Arch. Otolaryngol. Head –Neck Surg.,118:392-396.
- Brunton , and Pichinchero, M.E. (2005). Acut otitis media influence of the pcv - 7 vaccin on changes in the disease and it is management J fam pract .
- Buchman, C.A.;Doyle, W.J.and Pilcher, O. (2002). Nasal and otological effects of experimental respiratory syncytial virus infection in adults. A mJ otolar Berman,S.(1995). Manaagmentof acute and chronic otitis media in Pediatric Practice. Curr. Opin. Pediatric., 7:513-522

- Callender, M.D. (1992). Tuberculous otitis media . Report of a case Arch Otolaryngol. 25:11-16.
- Catlin, B.W. (1990). *Branhamella catarrhalis* . An orgaism ganing respect as a pathogen . Clin . Microbial. Rev.3:293-320.
- Chart, H. (1998). Toxogenic *E. coli*. J. of Applied Microbiology. ,84:77-86 .
- Chart, H. and Tenkin, S.(1999).Serodiagnosis of infection with verotoxin-producing *E. coli* .J. of Applied Microbiology ,86:731-740.
- Chigbu, C. O. and Ezeronye, O. V. (2003). Antibiotic resistant *Staphylococcus aureus*. *Afr. Bacteriol.* 2(10):374-378 .
- Chin, A. E.; Hedberg, K.; Cieslak, P. R.; Cassidy, M.; Stefonek, K.R. and Fleming, D. W. (1999). Tracking drug-resistant *Streptococcus pneumoniae* in oregon: an alternative surveillance method. *Emerging Infect. Dis.* 5(5).
- Chivez, M.; Lipez, J. L.; Coronilla, J.; Valverde, A.; Serrano, M. C.; Claro, R. and Mazuelos, E. M. (2002). Evaluation of the new VITEK2 system for determination of the susceptibility of clinical isolates of *Streptococcus pneumoniae*. J. Chemotherapy. 48:26 – 30.

- Chonmaitree ,T. ;Owen, M.J. and Patel, J.A.(1992). Effect of viral respiratory tract infection on outcome of acute otitis media. *J.pediatrics*,120(6):856-862
- Cimolai, N. and Adderley, R.Y.(1989) B.C. bacteremia in children . *Acta paediatr . scand* 78: 465-468
- Claes, J;Van-de-Heynyn, P.H. and Creten, W.(1992). Side effects of repeated otological application of fosfomycin in an animal study. *Arch. otolaryngology. Head-Neck Surg*, 118:951-955.
- Collee, J. G.; Fraser, A. G.; Marmino, B. P. and Simons, A. (1996). Mackin and McCartney Practical Medical Microbiology. 14th ed., The Churchill Livingstone, Inc. U.S.A
- Compagnari, A.A.; Shanks, K.L. and Dyer, D.W.E.(1994) Growth of *Moraxella catarrhalis* with human transferrin and lactoferrin expression of iron repressible proteins without siderophore production. *Infect. immun.* 62: 4906-4914.
- Cowan, A.S.T.(1985). Cowan and Steel's manual for identification of medical bacteria .2nd ed. London , Cambridge University Press ,UK
- Cruckshank, R.; Daguid, J.P.; Marmion, B.P. and Swain, R.H.A. (1975). Medical microbiology The English language book society London. vol. 2(12 th ed) P:459.

- Cullman, W.(1997) M.C.:virulence and resistance mechanisms
J.med.Microbe.,92(3):162-166.
- Dale, S. E.; Doherty-Kirby, A.; Lajoie, G. and Heinrichs, D.
E.(2004).Role of siderophore biosynthesis in virulence of
Staphylococcus aureus:Identification and characterization
of genes involved in production of a siderophore
Infect.Immun. 72 (1): 29 - 37.
- Dangan, R.;Fliss, D.M. and Einhorn, M.(1992). Outpatient
management of chronic suppurative otitis media without
cholesteatoma in children.Pediatr.Infect.dis.J.,11:542-546
- Deretic, V.;Dikshit,R.;Konyecsni,W.M.;Chakrabarty,A.M.and
Misra, T.K. (1989). The algR gene, which regulates
mucoidy in *Pseudomonas aeruginosa*, belongs to a class
of environmentally responsive Genes.J Bacteriol.171(3):
1278-1283.
- Dincer, A.D.; Takeli,A.; Ozturk, S. and Turgut, S.(1992).
Microorganisms isolated from chronic suppurative
otitis media and antimicrobial sensitivities.Microbiology
Bultemi,20:131-138
- Dinges, M. M.; Orwin, P. M. and Schlievert, P. M. (2000).
Exotoxins of *Staphylococcus aureus*.*J.Clin. Microbiol.*
13(1):16–34
- Donna, D. M.; Enoch, F.; Michael, G. and Russel, S. W. (2002).
Nasopharyngeal carriage and susceptibility patterns of

- Streptococcus pneumoniae* in Kumasi. Ghana. *West Afr. Med.* 21(3):233–236 .
- Drechsel, H.; Winkelmann, G. and Carrano, C.J. (1997). In *Transiti Metalsin Microbial Metabolism* (Harwood Academic)
- Duguid, J.P.; Anderson, E.S. and Campbell, I. (1996). Fimbriae and adhesive properties in *Salmonella*. *J Pathol Bacteriol.* 92:107-138.
- ElShamy, H.A. (1993). Bacteriology of chronic scretory otitis media in children. *J. Egypt an public health association*, Lxv III; 495-505.
- Epissito, S.; Noviello, O.S.D.; Errico, G. and Monthanaro, P.(1992). Topical ciprofloxacin VS: Intramuscular gentamycin for chronic suppurative otitis media. *Arch. otolaryngology. Head-Neck Surgery*, 118:842-844.
- Erkan, M.;Sevuk, E.; Asland, T. and Guney, E.(1994). Bacteriology of chronic suppurative otitis media. *Ann . otol. Rhinol. Laryngol.* 103:771-774
- Faden, H. ;Harabuchi, Y.and Hong,J.J. (1994) Epidemiology. of M.C. in children during first 2 years of life Relation ship to otitis media .*J infect.Dis* 169:1312- 1317
- Facklam, R. and Pigott, N. (1994). Description of phenotypic characteristics to aid in the identification of *S pneumoniae*.. In: Totollian, A. *Pathogenic Streptococci: present and future*. Lancer Publications, St. Petersburg,Russia415-417.

- Finkelstein, J.A.; Stille, C.J.; Rifas-Shiman, S.L. and Goldmann, D. (2005). Watchful waiting for acute otitis media: are parents and physicians ready? *Pediatrics* .115(6): 1466-1473 .
- Fireman, P. (1997). Otitis media and eustachian tube dysfunction connection to allergic rhinitis. *J. Allergy Clin. Immunol*, 99:5787-5797.
- Foster, T.J. and McDevitt, D. (1994) Molecular basis of adherence of Staphylococci to biomaterials. p. 31, In Bisno AL, Waldvogel FA (eds): *Infections Associated with Indwelling Medical Devices*, 2nd Edition. American Society for Microbiology, Washington, D.C.
- Francis, J.; Grimes, S.; Spradbrow, P. and Bensink, Z. (2002). A basic laboratory manual for the small scale-production and testing experimental. *FAO*, St. Lucia, Australia
- Gates, G.A. (1994). Current therapy in otolaryngology head and neck surgery . fifth edition . *Gates (mosby) London*. 11-14
- Gimmestad, M.; Sletta, H.; Ertesvag, H. and Bakkevig, K. (2003). The *Pseudomonas fluorescens* AlgG Protein, but Not Its Mannuronan C-5- Epimerase Activity, Is Needed for Alginate Polymer Formation. *J. Bacteriol.* 185:3515-3523
- Goebel, W.F. (1963). Colanic acid. *Proc. Nat. Acad. Sci. U.S.A.* 49:465-471 .
- Goel, U.K. and Kapil, A. (2001). Monoclonal antibodies against the iron regulated outer membrane

- proteins of *Acinetobacter baumannii* are bactericidal
B.M.C.Microbiol 1:16.
- Goodier, M.R. and Londei, M. (2000).Lipopolysaccharide stimulates the proliferation of human CD56+CD3- NK cells: a regulatory role of monocytes and IL-10. J. Immunol 165:139-147.
- Gorbach ,S.L.; Bartlett ,J.G, and Blacklow. (1998). Infectious diseasessecond edition. W.B.sauners company P530- 550.
- Goven, J.R.W.(1996).Pseudomonas , Stenotrophomonas. Burkholderia.Mackie and McCartney practical medical microbiology , 14th ed ., Churchill Livingstone,USA
- Gray, G.L.; Smith, D.H.; Baldrige, J.S.; Harkins, R.N.; Vasil, M.L.;Chen, E.Y. and Heyneker, H.L. (1999).Cloning, nucleotide sequence, and expression in Escherichia coli of the exotoxin A structural gene of Pseudomonas aeruginosa.Proc. Nat. Acad. Sci. USA 81:2635-2649
- Greene, A.M.D. (2002). Allergy of middle ear infection. Pediatr infect. Dis .J 17:(10) 3- 6 .
- Green wood, D.; Slack, R.C.; Penhetherer, J.F.(2002). medical Microbiology,Aguid to microbial infections, pathogenesis, immunity, laboratory diagnosis and control 16thed. Churchilllivingstone
- Griffiths, E.; Bullen, J.J. and Griffiths, E. (1987). ed. In Iron and Infection (Wiley. Chichester. U.K.)

- Gums, J. G. and Gainesville, F. L. (2000). *Streptococcus pneumoniae* susceptibility from 1995 – 2000: results of the Antimicrobial Resistance Management (ARM) program. *Antimicrobiol. Chemother.* 70: 730 – 731.
- Hacker, J.; Blum-Oehler, G.; Muhldoreff, I. and Tschape, H. (1997). Pathogenicity islands of virulent bacteria: Structure, Function and Impact on microbial evolution. *Mol. Microbiol.* 23: 1089 - 1097.
- Hancock, R.E. and Spreet, D.P. (2000). Antibiotic resistance in *P. aeruginosa*: mechanism and impact on treatment. *Drug Resistance updates*, 3: 247-255
- Hanniffy, S.B.; Carter, A.T.; Hitchin, E. and Wells, J.M. (2007). Mucosal *Lactococcus lactis* Pneumococcal protection against vaccine using affords respiratory infection *Dis. J. Infect.* 195: 185-193.
- Hanumanthappa, A.R.; Chandrappa, N. R., Rajasekharappa M.G. (2003). Prevalence of *methicillin resistant Staphylococcus aureus* in Karnataka. *Indian J. Pathol. Microbiol.*, 46(1): 32-129.
- Harabuchi, Y.; Faden, H. and Yamanaka, N. (1994). Nasopharyngeal colonization with nontypeable *Haemophilus influenzae* and recurrent otitis media. *J. Infect. Dis.*, 170: 862-866.

- Heikens, E.A.; Fleer, A.H.; Flor, D.A. and Pavw N.B.(2005).
Comparson of genotypic and phenotypi methods for
identification of Coagulase-negative Staphylcoccus
. J. Clin. Microb., 43:2286-2290.
- Heikkinen, T. ;Thint, M. and Chonmaitree, T. (1999)
Prevalence of various respiratory viruses in the middle
ear during acute otitis media. N Engl J Med . 28; 340(4):
260-264
- Holt, J. C.; Krieg, N. R.; Sneath A.; Stachley, J. T. and
Williams,S. T. (1994). Bergy's manual of determinative
bacteriology.9thed., U.S.A.P. 552 .
- Hornick, D. B.; Allen, B. L.; Horn, M. A. and Clegg, S. (1999).
Adherence to respiratory epithelia by recombinant
Escherichia coli expressing *Klebsiella pneumoniae* type 3
fimberial gene products. *Infect. Immun.* 60: 1577 – 1588.
- Ibekwe, A.O., AlShareef,Z.and Benaayam, A. (1997). Anaerobes
and Fungi in chronic suppurative otitis media. J.
Reprinted from Annals of Otology, Rhinology, and
Larynology.V106(8):649-652.
- Iglewski, B.H. (1996). *Pseudomonas*. In: Baron's Medical
Microbiology (*Barron S et al, eds.*), 4th ed., Univ of
Texas Medical Branch.
- Ikeh,E.I.;Adebayo,E.O.; Okuonghae,H.O. and Ighagboja,L.S.
(1993). Bacteriology of chronic discharging ears ichildren
of Jos, Northern Nigeria. Nigeria medical Practitioner,26:1-4

- Ilicali , O.; Keleş, N.; Deger, K.; Savaş, I. (1999). Relationship of passive cigarette smoking to otitis media. *Arch. Otolaryngol Head Neck Surg* 125 (7): 758-762
- Imamura, Y. ; Higashiyama, Y.; Tomono, K.and Izumikawa, K. (2005).Azithromycin Exhibits Bactericidal Effects on *Pseudomonas aeruginosa* throughInteraction with the Outer Membrane.Antimicrobial Agents and Chemotherapy, Vol. 49, (. 4) p. 1377-1380 .
- Indudharan, R.; Hag, J.A. and Aiyar, S.(1999). Antibiotics in chronic suppurative otitis media a Bacteriology study . *Ann.Otol.Rhinol.Laryngol.*,108:440-445
- Jain, S. and Ohman ,D.E.(1998). Deletion of algK in mucoid *Pseudomonas aeruginosa* Blocks Aliginate polymer formation and Bacterial results in uronic acid secretion *J:180(3).*634-641
- Joseph, W. and St Geme, I. I. (2002). Molecular and cellular determinants of non typeable *Haemophilus influenzae* adherence and invasion. *CellularMicrobiology.*4(4):191
- Karalus, R. and Compagnari, A.(2000).M.C. areview of an important human mucosal pathogen . *Microbes, infect.* 547-559.
- Karlowsky,J.A.;Draghi,D.C.;Jones,M.E.;Thornsberry,C.;Friedla -nd,I.R.and Sahm,D.F.(2003). Surveillance for Antimicrobial Susceptibility among clinical isolates of

- Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States, 1998-2001.
- Karlsson, A. and Arvidson, S. (2002). Variation in extracellular protease production among clinical isolates of *S.aureus* due to different levels of expression of the protease repressor *SarA*. *Infect. Immun.* 70 (8): 4239 – 4246.
- Karlsson, A.; Saravia-Otten, P.; Tegmark, K; Morfeldt, E. and Arvidson, S. (2001) Decreased amounts of cell wall-associated protein A and fibronectin-binding proteins in *Staphylococcus aureus* *SarA* mutants due to up-regulation of extracellular protease. *Infect. Immun.* 69: 4742 – 4748
- Kenna, M.A. (1994). "Treatment of chronic suppurative otitis media. Otolaryngology". *Clin. North Am.* 27:457-472.
- Kenneth, T. (2002). Bacteriology, *Ps. aeruginosa*. University of Wisconsin-Madison p330,
- Kilian, M. (2002). *Streptococcus* and *enterococcus*. In Greenwood D., Slack R.C.B., Peutherer J.F. *Medical Microbiology*. 60th ed. Churchill Livingstone. p 174
- Kirby, R. and Ruoff, K.L. (1995). Cost effective clinically relevant method for rapid identification of beta hemolytic *Streptococci* and *Enterococci*. *J.Clin. Microbiol.*, 33:1154-1157
- Kharazmi, A.G.; Doring, N.; Hgiby and Valerius (1984). Interaction of *Ps.aeruginosa* alkaline protease and elastase with

- human polymorphnuclear leukocyte in vitro. *Infect. Immune.* 43: 161-165
- Klein, J.O.; Bennett,J.E. and Dolin, R.(2000). Principles and practico of infectious diseases .fifth edition.Churchill livingstone.Vol1p.669-676
- Klingman, K.L.; Pye, A. and Murphy, T.F. (1995). Hill: Dynamics of respiratory tract colonization by *Branhamella catarrhalis* in bronchiectasis .*AmJ Respir. Crit care .Med* 152:1072-1078 .
- Kloss ,W.E. and Bannerman, T.L.(1994). Up date on clinical significance of coagulase negative *Staphylococci*. *Cli. Microbiolo.Rev.*7(11):117-120
- Krohn, M. A.; Hillier, S. L.; Bell, T. A.; Kronmal, R. A. and Grayston, J. T. (1993). The bacterial etiology of conjunctivitis in early infancy: Eye prophylaxis study group. *Am. J. Epidemiol.* 138 (5): 326 – 332
- Lee,K.J. (2003). Essential otolaryngology head and neck surgery . eighth edition.MacGrw-Hill.473-511.
- Legent, F.; Bordure, P. and Beauvillain,C.(1994). Controlled prospective Study of oral ciprofloxacin versus amoxcillin, clavulonic acid in chronic supplicative otitis media in adults. *Chemotherapy*,40: 16-23.
- Lim, D. J. Mogi, G. (1994.) Mucosal immunology of middle ear and Eustachian tube. In Ogra P.L.; Mestecky, J.;

- Lima, A. L.; Belford, R. J.; Moeller, C. T.; Branco, B. C.; de Sousa, L. B. and Freitas, D. (2004). In vitro antibiotic susceptibility of ocular bacteria isolates from the cornea and conjunctiva to moxifloxacin, gatifloxacin and other fluoroquinolones. *Arq. Bras. Ophthalmol.* 67(6):883 – 886
- Little, P.; Moore, M.; Warner, G.; Dunleavy, J. and Williamson, I. (2006). Longer term outcomes from a randomised trial of prescribing strategies in otitis media. *Br J Gen Pract* 56 (524):176-182 .
- Lysenko, E.S.; Ratner, A.J.; Nelson, A.L. and Weiser, J.N. (2005). The role of innate immune responses in the outcome of interspecies competition for colonization of mucosal surfaces. *PloS Pathog* 1 (1)
- MacFaddin, J.F. (2000). Biochemical tests for the identification of medical bacteria. 3rd ed., The Williams and Williams and Wilkins-Baltimore, U.S.A.
- Macnamar, M.; Phillips, D. and Proop, D.W. (1996). The bone anchored hearing aid in chronic suppurative otitis media (CSOM). *J. Laryngol. Otol.* 110:38-40.
- Maeda, H. and Yamamoto, T. (1996). Pathogenic mechanisms induced by microbial proteases in microbial infection *Biol. Chem. Hoppe Seyler.* 377(4): 216-226
- Mak, D.; Dipr, A.; Munchendrick, A.; Weeks, S. and Plant, A. (2000). Middle ear disease in remote aboriginal Australia a field

assessment of surgical outcomes..of Lary.and Otol.114
(1):26-32.

- Marchetti, F.;Ronfani, L.;Nibali,S.;and Tamburlini, G.(2005).
Delayed prescription may reduce the use of antibiotics
for acute Otitis media: a prospective observational
study in primary care. *Arch Pediatr Adolesc Med* 159
(7): 679-684.
- Martinez, B. O.; Ruiz, R. M. and Perez, R. M. (2004). Bacterial
prevalence of most pathogens and susceptibility to
antibiotics.*Antimicrob.Agents Chemother.*61(1):32–36.
- Martino, P.; Liverlli, V.; Sirot, D.; Joly, B. and Darfeuille, A.
(1996)A new fimbrial antigen harbored by CAZ-5/SHV-4
producing *Klebsiella pneumoniae* strains involved in
nosocomial infections. *Infect. Immun.* 64: 2266 – 2273
- Masalha, M.; Brovok, I.; Schreiber, R.; Aharonowitz, Y. and
Cohen, G. (2001). Analysis of transcription of the
Staphylococcus aureus aerobic class Ib and anaerobic
class III ribonucleotide reductase genes in response to
oxygen. *J. Bacteriol.* 183 (24): 7260 – 7272
- Masters, P. J. (1997). The role of Beta-lactamase in the use of
Beta-lactam antibiotics. *J. Microbiol. Infect.*
Dis. 26 (1): 556 – 557.
- Mazmanian, S. K.; Skaar, E. P.; Gaspar, A. H.; Humayun,
M.; Gornicki, P.; Jelenska, J.; Joachmiak, A.;
Missiakas, D. M. and Schneewind, O. (2003).

Passage of heme-iron across the envelope of
Staphylococcus aureus. *Science*. 299: 906 – 909

McDaniel and Larry (2005). Gram positive cocci . university
of Mississippi Medical center

Mellenkvist,A.; Nordst, T.; Hallden, C.and Christenses, J.J
(2003).The *Moraxella catarrhalis* Immunoglobulin
D-Binding protein MID has conserved sequences
and is regulated by a mechanism corresponding to
phase variation. *Journal of bacteriology*, Vol. 185,
(7). P. 2285-2295

Miniatis, T.; Fritsch, E. and Sambrook, J. *Molecular cloning, a*
(1982).Laboratory Manual. Cold Spring Harbour
Laboratory.NewYork

Miyata, S.;M. Casey, D. W.; Frank, F. M. and Ausubel, .
as (2003), Use of the *Galleria mellonella* caterpillar
a model host to study the role of the type III
secretion system in *Pseudomonas aeruginosa*
pathogenesis. *Infect. Immun.* 71:2404-2413

Mouricout, M.(1997). Interactions between the enteric pathogen
and the host. An assortment of bacterial lectins and a set of
glycoconjugate receptors. *Adv. Exp. Med. Biol.* 412:109-123

- Moxon, E. R. and Murphy, T. F. (2000). *Haemophilus influenzae*. In: Mandell, G. L.; Bennett, J. E. and Dolin, R. Mandell, Douglas and Bennett's principles and practice of infectious diseases. 5th ed., New York: Churchill Livingstone. 2369 - 2378.
- Murray,P.R.; Jorgensen, J.H.; Pfaller, M.A.;Yolen, R.H. (2003). Manual of clinical Microbiology 8th ed., Washington,D.C. Vol 1 .
- Musher, D. M. (1992). Infections caused by *Streptococcus pneumoniae*: Clinical spectrum, pathogenesis, immunity and treatment. *Clin. Infect. Dis.* 14: 80-809.
- Murphy, T.F.(1996)B.C.Epidemiology, surfaceantigenic structure and immune response: Microbial . Rev.J. 60 (2): 267-279.
- Nair, S. P.; Williams, R. J. and Henderson, B. (2000). Advance in our understanding of the bone and joint pathology caused by *Staphylococcus aureus* infection. *Rheumatology*. 39: 821 - 834.
- Nassif, X.;Fournier, J.;Arndel, J.and Sansonetti, P.(1989). Muroid phenotype of *Klebsiella pneumoniae* is a plasmid encoded virulence factor. *Infect. Immun.* 57 (2): 546 - 552
- Nichol, K. A.; Zhanel, G. G. and Hoban, D. J. (2003). Molecular epidemiology of Penicillin-Resistant and Ciprofloxacin-Resistant *Streptococcus pneumoniae* in Canada.*Antimicrob. Agents Chemother.* 47 (2): 804 – 808

- Nizet ,V.;Colina, K.F. and Almiquist J.R.(1996). A virulent non encapsulated *Haemophilus influenzae* . J.Infect.Dis.,173(19):180-186
- Novak, R.; Brown, J. S.; Charpentier, E. and Tuamonen, E. (1998).Penicillin tolerance genes of *Streptococcus pneumoniae* the ABC type manganese permease complex Psa. *Mol. Microbiol.* 29: 1285 – 1296.
- O'Hara, C.M. ; Brenner, F.W. and Miller J.M.(2000). Classification, identification and clinical significance of *proteus*, *Providencia* and *Morganella*. Clinical microbiology reviews,13:534-546.
- Perez,J.L.;Pulido,A.;Gomez,E.;Sauca,G.and Martin,R.(1990) . Superoxol and anino peptidase tests for identification of pathogenic *Neisseria* species and *Moraxella (Branhamella) catarrhalis* . Eur.J.Clin. Microbiol Infect. Dis.9(6): 421-423
- Peterson, L.R. (2006). Penicillins for treatment of pneumococcal pneumonia: does in vitro resistance really matter. *Clin.Infe.Dis.*42(2):224-233.
- Piret, J.; Millet, J. and Demain, A. (1983). Production of intracellular protease during sporulation of *Bacillus brevis*. *Eur. J. Appl. Microbiol. Biotechnol.* 17: 227-230
- Pitkaranta, A.; Virolainen, A.and Jero, J. (1998) .Detection of, rhino virus respiratory syncytial virus, and corona virus

infection in acute otitis media by reverse transcriptase polymerase chain reaction *pediatrics*,102:291-295.

Podschun, R.; Fischer, A. and Ulmann, U. (1992). Siderophore production of *Klebsiella spp* isolated from different sources. *Zentbl. Bacteriol.* 276: 481 - 486

Podschun, R.; Penner, I. and Ulmann, U. (1992). Interaction of *Klebsiella* capsule type 7 with human polymorphonuclear leucocytes. *Microb. Pathol.* 13: 371 - 379.

Podschun, R. and Ulmann, U. (1998). *Klebsiella spp* as nosocomial pathogens: Epidemiology, Toxonomy, Typing methods and Pathogenicity factors. *Clin. Microbiol. Rev.* 11 (4): 589 - 603.

Podschun, R.; Pietsch, S.; Holler, C. and Ulmann, U. (2001). Incidence of *Klebsiella spp* in surface waters and their expression of virulence factors. *Appl. Environ. Microbiol.* 67: 3325 - 3327.

Poulsen, K.; Reinholdt, J. and Kilian, M. (1996). Characterization of the *Streptococcus pneumoniae* immunoglobulin A1 protease gene *iga* and its translation product. *Infect. Immun.* 64: 3957 – 3966.

Podoshin, L.; Frodis, M. and David, J.B.(1989). Ototoxicity of ear drops in patients suffering from chronic otitis media. *J. Laryngol.Otol.* 103:46-50

- Prithiviraj, B.; Bais, H.; Weir, T.; Suresh, B.; Najarro, E.; Dayakar, B.; Schweizer, H. and Vivanco, J. (2005). Down regulation of virulence factors of *Pseudomonas aeruginosa* by salicylic acid attenuates its virulence on *Arabidopsis thaliana* and *Caenorhabditis elegans*. *Infect Immun* 73 (9): 5319-5328
- Proctor, M. and Manning, P. J. (1999). Production of immunoglobulin A protease by *Streptococcus pneumoniae* from animals. *Infect. Immune*. 58 (9): 2733 – 2737.
- Rae, M.B.; Tanksale, A.P. ; Ghathge, M.S. and Deshpande, V.V. (1998).Molecular and biotecnological aspects of microbial proteases.*Microbiology and Molecular biology Reviews*. 62(3):596-635.
- Rahme, L.; Stevens, E. ; Wolfort, S. ; Shao, J. ; Tompkins, R. and Ausubel, F. M. (1997). Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 268:1899-1902.
- Reinholdt, J. and Kilian, M. (1997). Comparative analysis of immunoglobulin A1 protease activity among bacteria representing different Genera, Species and Strains. *Infect. Immun*.65(11):4452-4459.
- Raksha, R.; Jone, M. ; Cappello, F. and Garicia, S.(2003). Comparative analysis of the roles surface proteases enzymes in virulent *Staphylococcus* strains. *J. Infect. Dis*. 56: 1234-1240.

- Rashmi S.; Lal S. C. and Bhumneshwar K. (2005). Antibacterial resistance: current problem and possible solution. Indian J. Med. Sci. 59: 120-129.
- Ratto, M. (2006) Colanic acid is an exopolysaccharide common to many enterobacteria isolated from paper-machine slimes. *J. of Industrial Microbiology & Biotechnology* 33(5)
- Reckville, P. and Bethesd, M.D. (2006) . secretory otitis media . Jotolaryngol head and neck surgery , 23 suppl 2: 37 -42 .
- Robbins, J.B. ; Schneerson, R. and Szu, S.C.(1988). Recent development of bacterial vaccines related to the prevention of otitis media . In Recent A dvances in otitis media .edited by D.J,Lim.Philadelphia:B.C.Decker.Pp.210-214.
- Rubens, C.E., M.R. Wessels *et.al.*, (1987).Transposon mutagenesis of typeIII GBS, correlation of capsule expression with virulence. Proc Natl Acad Sci USA. 84:7208-12.
- Ryan, K.J. and Ray, C.G. (editors) (2004). *Sherris Medical Microbiology*,4thed.,McGrawHill .
- Sauer, F.G.; Mulvey, M.A. ;Schilling, J.D. ; Martinez J.J., and Hultgren, S.J.(2000).Bacterial pili: molecular mechanisms of pathogenesis.*Curr.Opin.Microbiol.*3:65-72.
- Schennings, T.; Heimdahl, A.; Coster, K. and Flock, J. (1993). Immunization with fibronectin binding protein from *Staphylococcus aureus* protects against experimental endocarditis in rats. *Microb. Pathol.* 15: 227 – 236.

- Schneewind, O.; Fowler, A. and Faull, K. F. (1995). *Staphylococcus aureus* infections. *Science*. 268: 103 - 106.
- Schweizer, H.P.(1998). Intrinsic resistance to inhibitors of fatty acid biosynthesis in *P.aeruginosa* is due to efflux: application of a novel technique for generation of unmarked chromosomal mutation for the study of efflux systems *J. antimicrob.agents Chemother.*, 42:394
- Scott-Brown's otolaryngology, otology.(1987).Edited by Both,J.B. fifth edition.vol.3,203-227.
- Scott-Brown's otolaryngology, otology.(1997).Edited by Kerr, A.G. ,Adams, D.A. and Cinnamond, M.J sixth edition.vol.3.
- Senior, B.W.and Sweeney, G.(1984).The association of Particular types *Proteus* with chronic suppurative otitis media. *J. of medical Microbiology*. :17, 201-205.
- Shenio, P.M.(1987). Management of chronic suppurative Otolaryngology. 5th ed., Butter's otitis media. Scott-Brown Worth,London,Vol.3,P:215-237 .
- Shimotakahava,S.G.;Ruby,R.F.and Lanpe,H.B.(1989). Otitis media with effusion in the adult.*J. of Otol. Rhino. and Lary*.18(3):85-88.
- Shurin, P.A.;Giebink, G.S.;WEgman, D.L. ; Ambrosino, D.; Rholl, J.; Overman, M.;Bauer, T. and Grsiber, (1988). Prevenation of pneumococcal otitis media in Chinchillas with human bacterial polysaccharide immunoglobulin. *J.list Clin Microbiol* 26 (4):755-759.

- Smith ,H.R. and Scotland S.M.(1993). Isolation and identification methods for *E.coli* O157 and other verocytotoxic producing strain. J. Clin. Pathol., 46:10-17.
- Soh, S. W.; Poh, C. L. and Lin, R. V. (2000). Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolated from pediatric patients in Singapore. *Antimicrob. Agents Chemother.* 44: 2193 – 2196.
- Sorensen, R.U.; Klinger, J.D.; Cash, H.A.; Chase, P .A; and Dear Dorn, D.G. (1983). In vitro inhibition of lymphocyte proliferation by *Ps. aeruginosa* phenajin pigments. *Infect. Immune.* 41: 324 - 330.
- Stenfors, L. E. and Raisanen, S. (1992). In vivo attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* to Nasopharyngeal epithelium in children. *Int.J. pediatr. Otolaryngology.*,54:25-28 .
- Stenfors, L. E. and Raisanen, S. (1993). Opsonization and phagocytosis of bacteria during various middle ear infections. *Int.J.*
- Stenfors, L.E. (1999). Nonspecific and specific immunity to bacterial invasion of the middle ear cavity. *Int. J. Pediatr. Otorhinol .* 49 (suppl. 1) 5223 -5226.
- Stevens ,D.;Lieberman,M.; McNitt,T. and Price,J. (1984). Demonstration of uronic acid capsular material in

- mucoid *Pseudomonas aeruginosa*. J Clin Microbial. 19(6):942-943 .
- Sweeney, G.; Picozzi, G.L. and Browning, G. (1982). A quantitative study of aerobic and anaerobic bacteria in chronic suppurative otitis media. Journal of infection:5, 47-55.
- Tai, S. S.; Lee, C. J. and Winter, R. E. (1993). Hemin utilization is related to virulence of *Streptococcus pneumoniae*. *Infect. Immune*. 61: 5401 – 5404.
- Tarlow , M. (1998). Otitis Media Pathogenesis and Medical sequelae. *Ear.Nose.Throat*. 77(6supple):3-6.
- Todar, K., (1998). Staphylococcus in Bacteriology.
- Todar, K., (2003). Streptococcus in Bacteriology.
- Todar, K., (2004). Pseudomonas in Bacteriology.
- Todar, K., (2004). Haemophilus in Bacteriology.
- Tonkic M.; Barisic I.G.; and Punda – Polic V. (2005). Prevalence and antimicrobial resistance of extended-spectrum β -Lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* strains isolated in a university hospital in Split, Croatia. *Int. Microbiol.* 8: 119-240.
- Tuomanen, E. I.; Austrian, R. and Masure, H. R. (1995). Pathogenesis of pneumococcal infection. *N. Engl. J. Med.* 332: 1280.
- Varon, E.; Levy, C.; Dela Rocque, F. and Boucherat, M. (2000). Impact of antimicrobial therapy on nasopharyngeal

- carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Branhamella catarrhalis* in children with respiratory tract infections clin. Infect. Dis. 31:477-481.
- Venegas, M. F.; Navas, E. L.; Gaffney, R. A. and Duncan, J. L (1995). Binding of type 1 piliated *Escherichia coli* to vaginal mucus. *Infect. Immun.* 63:416 – 422
- Walker, T. S.; Bais, H. P. ; Déziel, E. and Schweizer, H. P. (2004). *Pseudomonas aeruginosa*-plant root interactions. Pathogenicity, biofilm formation, and root exudation. *Plant Physiol.* 134:320-331 .
- Wallace, M.R. and Old field, E.C.(1990)III : M.C. bacterum Arch. Intern. Med 150:1332-1334 .
- Weber, K .and Osborn, M. (1982). The reliability of molecular weight determination by dodeyl sulfate -polyacrylamide Gel electrophoresis. *In physical Biochem.*
- Wilkinson, J.F.; Dudman, W.F. and Aspinall, G.O.(2007) The extracellular polysaccharide of *Aerobacter aerogenes* A3 (S1) (*Klebsiella* type 54). *Biochem J.* ;59(3):446–4451.
- Wizemann, T. M.; Adamon, J. E. and Langermann, S. (1999). Adhesions as targets for vaccine development. *Emer. Infect. Dis.* 5 (3): 395 – 401.
- Yarwood, J. M. and Schlievert, P. M. (2000). Oxygen and carbon dioxide regulation of toxic shock syndrome toxin I

production by *Staphylococcus aureus* MN8. *J. Clin. Microbiol.* 38: 1797 – 1803.

Yassin ,H.(1990). Chronic otitis media microbiological and epidemiological study Master thesis, College of Medicin, university of Basrah .

Yilmaz, T.;Ceylan, M.; and Akyon, Y. (2006).Helicobacter pylori: a possible association with otitis media with effusion. *Otolaryngol Head Neck Surg*134(5):772-777 .

Yuen ,P. W.; Lauos, K.and Chau, P.Y.(1996). Ofloxacin ear drop treatment for active chronic suppurative otitis media ; prospective randomized study. *Am.J. otol* . 15 : 670-673.